/*----------*/
Page: 5

Date:. 4/10/2014

Title:. Overnight cultures

Purpose:. To make overnight cultures for strains J53-1 of *E. coli* k12 harboring plasmid pDU1358 for -80^{0}C stocks. Strain was received from Anne O. Summers, University of Georgia

Methods:.

Results:.Order form

Conclusion:.

/*----------*/
Page: 6

Date:. 4/10/14

Title:. Continuation from pg 5

Purpose:.

Methods:.
Apply sterile technique. All steps close to a flame.

- Apply a few drops of 70% ethanol to the bend, as well as your hands. Swipe bench
- Ignite bunsen burner
- Acquire 5ml pipette tubes
- Acquire LB media botte 5 sterile test tubes
- Open bottle lid using index and middle fingers, hold bottle at 45^0 angle and flame the opening
- Run the 5ml pipette through the flame
- Pull up 5ml of LB into pipette. close bottle
- Hold the lid of a test tube with your palm and little finger
- Flame the tube opening . Run the pipette through the flame
- Release 5 ml of LB into test tube
- Flame test tube opening
- Replace the lid of the test tubes
- Repeat 4 more times. discard 5 ml pipette in designated container
- Acquire Km (Kanamycin) 50 mg/ml stock from the fridge
- Using a micropipette, obtain 5 ul of Km

- Flame the opening of one of the LB-containing test tubes. Insert the tip of the pipette tip.
- Tip the tube at an 80^0 angle so that the medium touches the pipette tip
- release the Km into the medium
- flame the test tube opening. Replace lid
- Discard the pipette tip repeat 4 more times.
- Shake a bottle of swabs so that you may obtain one without touching the others
- Close the tube
- Pick an isolated colony
- Flame the opening of a test tube. Shake swabe in LBN to release bacteria. Fame tube and replace lid.
- Repeat 4 more times.
- Place tubes 37^0C incubator

Results:.

Conclusion:.

/*----------*/
Page: 7

Date:. 4/11/2014

Title:. Creating -80 stocks of K12 *E. coli* strain harboring pDU1358

Purpose:.To create a stock of *E. coil* that can be revived at any time for plasmid extraction.

Methods:.
Add 300 ul of 50% Glycerol stock and 700 ul of overnight culture in LB and Km
Mix, remove air bubbles
Incubate in -80^0C fridge

Results:. Strain saved as 2954(JG)

Conclusion:. Strain containing plasmid pDU1358 was store at -80^0 C for further use.

/*----------*/
Page: 8

Date:. 4-11-2014

Title:. Purification of pDU1358 plasmid from *E. coli* strain

Purpose:. Purification of plasmid for storage at -20^{0}C using the Quick Plasmid Miniprep kit

Methods:.

- Sterilize using ethanol
- Light bunsen flame
- Pre-heat 300 ul of TE buffer (for large plasmids)
- Add 2mL of overnight culture to 2mL tubes
- Repeat for all 4 tubes
- Centrifuge for 1 minute at 1500 rpm (8000 x g)
- Remove supernatant
- Add 2ml of overnight culture to each of the tubes
- Repeat centrifugation. Make sure tubes are balanced with the edge facing outwards
- Acquire Resuspension buffer from fridge
- Add 250 ul of resuspension buffer & resuspend until homogeneous. Return R3.
- Add 250 ul of Lysis Buffer. Mix by inverting.
- Repeat for all 4 tubes
- Incubate for 5 minutes at room temp.
- Add 350ul of precipitation buffer
- Mix by inverting tube
- Centrifuge at 15000 rpm for 10 minutes
- load supernatant onto spin column in 2 mL wash tubes by tipping the tube and extracting using pipette (85ul)
- Centrifuge

Results:.

Conclusion:. Plasmids (4) were stored in iGEM 2014 Box in walk-in freezer 361.

/*----------*/
Page: 9

Date:. 4/11/2014

Title:. Primer Design to create a BioBrick part for mer operon

Purpose:. Design primers to amplify mer operon from pDU1358 (provided by Anne Summers) while simultaneously eliminating the EcoRI restriction site within merA.

Methods:.Obtained mer operon sequence from Aunica Kane
Scanned sequence for restriction sites conflicting with iGEM standard RFCIO.

Results:.

RE site	Location (bp, operon context)	Mutation
EcoRI	2243	A2245G

The primers used for the first PCR reaction will be:

Forward
GTCGCGCATGTCAACGGCGA**G**TTCGTGCTGACCACGGGACA
20 nt: A=4 T=3 C=5 G=8 CG=65.00%

Reverse
ACTAGTTCACGGTGTCCTAGATGACA
20 nt: A=5 T=5 C=5 G=5 CG=50.00%

Conclusion:. The first PCR reaction will be carried out to mutate the codon GAA into GAG. This codon will still code for glutamic acid,; however, it will alter the EcoRI restriction site present within merA sequence. The cgat code was added to the primers to al the polymerase to position onto the sequence.

/*----------*/
Page: 10

Date:. Papers

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 11

Date:. Papers

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 12

Date:. 4/17/2014

Title:. Making LB+ Km Agar media

Purpose:. To select colonies that harbor the correct plasmid, we will use kanamycin as a selection tool

Methods:.
Rinse 2L bottle with DI water
Add stir bar
Rinse 1 L Graduated cylinder
Add 1 L of MilliQ ddH_2O to bottle
Weigh 28.0 g of LB broth, pour into water
Stir at 500 rpm
Add 15.0 g of Agar to make 15% media
Autoclave at liquid 60

Results:.

Conclusion:.Liquid media was placed in 55^0C incubator

/*----------*/
Page: 13

Date:. 4/18/2014

Title:. Preparing LB + Km Agar plates coated with Xgal

Purpose:. Inoculate plates with Xgal so that *E. coli* containing lacZ may grow and exhibit a blue color. Plates used were LB+ Km

Methods:.

- Place a few drops of Xgal 30mg.mL on a plate for a total of 75 uL
- Dip a spreader in ethanol, flame it, allow it to cool for a few seconds (5-10)
- Spread the Xgal throughout the plate surface while rotating the plate
- Flip plates and allow them to settle so that the Xgal is incorporated into the media. Place plates in 37^0C incubator, if further drying is needed.

Results:.

Conclusion:.

/*----------*/
Page: 14

Date:. 4/21/2014

Title:.Transforming pBBR-BB into pUQ950 *E. coli* cells

Purpose:.To put the pBBR-BB plasmid into the strain of *Escherichia coli* pUQ950 in order to make a chassis for the mer operon BioBrick part and stock of *E. coli* with the pBBR-BB plasmid for future use.

Methods:.
"Quick" Transformation Protocol- For already circularized plasmids
- Turn on water bath and set to 42^0C water.
- Thaw UQ950 competent cells on ice.
- Add 0.5 uL of pBBR-BB DNA to competent cells.
- Incubate on ice for 15 minutes.
- Heat-shock@ 42^0C for 45 seconds; put back on ice for 5 min
- Add 750 uL of RB (Rubidium) medium
- Incubate @ 37^0C while shaking for 45 min.
- Make 1:10, 1:100, and 1:1000 serial dilution by pipetting 10uL of the cells into 90uL RB medium, gently mixing, removing 10 uL from the 1:10 dilution and adding 90 uL of fresh RB medium to produce a 1:100 dilution, and removing 10 uL of dilution and adding it to 90 uL of fresh RB medium to make 1:1000 dilution.
- Plate ~75uL of each dilution and the original concentration onto separate LB media plates with kanamycin using a spreader.
- Incubate the plates at 37^0C
- Purified pBBR-BB was placed in iGEM 20147 box in the walk-in freezer 361 at -20^0C.

Results:.

Conclusion:.

/*----------*/
Page: 15

Date:. 4/22/2014

Title:. PCR Amplification of merAB region

Purpose:.To amplify the 40 bp region of merA contain the EcoRI restriction site and merB to produce a double-stranded sequence of DNA that does not carry the restriction site.

Methods:.

- Thaw pDU1358 on ice from compartment 1 in iGEM 2014 Box
- Add 307 ul of nuclease-free (NF) H_2O to merR fwd primer tube
- Add 365 ul of NF H_2O to merB rev primer tube
- Add 374 ul of NF H_2O to merTPAB primer tube
- Add 90 ul of NF H_2O to each of 3 1.5 mL tubes
- Thaw 2x Go Taq green mix on ice
- Quantity pDU1358 (concentration 9.98 ng/ul)
- Obtain 4 PCR tubes
    - Add 25 uL green mix
    - 0.5 uL fwd primer (merTPAB)
        Tube 1 = 10uM (Basem)
        Tube 2 = 10uM (Jen)
        Tube 3 = 100uM (Basem)
        Tube 4 = 100 uM (Jen)
    - 0.5 uL rev primer (MerB rev)
        Tube 1 = 10uM (Basem)
        Tube 2 = 10uM (Jen)
        Tube 3 = 100uM (Basem)
        Tube 4 = 100 uM (Jen)
    - 1.0 uL pDU1358 (9.98 ng/ul)
    - 22.5 uL NF H_2O
    - 0.5 uL High fidelity polymerase

    Total = 50 uL Tap tubes and spin down
- Run PCR at

1	2	3	4	5	6	7
95^0C	95^0C	57^0C	72^0C	steps 2-4	72^0C	10^0C
2:00	0:30	0:30	1:30	28x	5:00	oo

Denature		Anneal	Extend		Final Extension	

Results:.

Conclusion:. This will be later used as the reverse primer in the PCR reaction of the entire mer operon. DNA used in PCR will be purified pDU1358 plasmid.

/*----------*/
Page: 16

Date:. 4/22/14

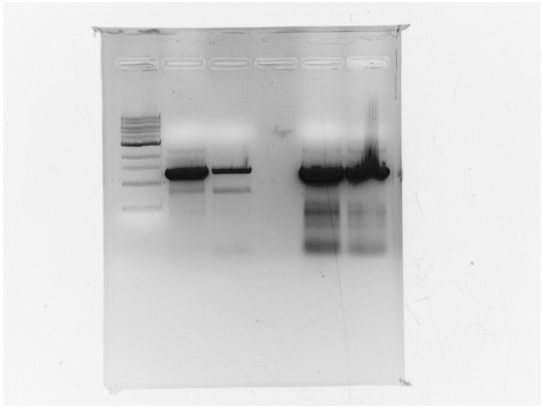
Title:. Prepare Gel to run PCR product

Purpose:. To prepare a gel on which the 4 product of the PCR reaction on pg 15 will be allowed to run, and the fragment containing merAB could be separated

Methods:.

- Obtain 1% Agar/Ethidium Bromide mixture from 55^0C incubator
- Rotate the well plate after wetting the sides so that the compartment is sealed off. Balance.
- Pour mixture into plate
- Place comb to create wells
- Allow gel to solidify
- Rotate plate back to original position, make sure gel is submerged
- Place 1 kb ladder and dye into first well
- In the 2nd well add the pcr product of tube 1 (refer to p. 15)
  3rd well add the pcr product of tube 2
  5th well add the pcr product of tube 3
  6th well add the pcr product of tube 4
- Run gel for 45 mins

Results:.



Conclusion:.

/*----------*/

Page: 17

Date:. 4/22/14

Title:. DNA Quantification Using the Qubit

Purpose:. To determine the DNA concentration of our purified plasmid stock of pDU1358

Methods:.

Use the Quant-It Broad Range kit

- Add 198 uL f Qubit dsDNA BR Buffer to clear-walled Qubit tube (located in the "Qubit Quantification" drawer beneath the Qubit machine.)
- Add 1 uL of pDU1358 plasmid DNA to the Qubit tube.
- Add 1 uL of Qubit dsDNA BR reagent 200x (dye) to the Qubit tube. Mix
- Place the Qubit tube in the Qubit fluorometer.
- Press any button to activate the screen and select the program for BR, then press "Go."
- Use the arrow buttons to select "Use Last calibration," and press "Go."
- After the machine calibrates and reads a quantification concentration, convert this concentration to the amount of your original loader sample (1 uL).
- Convert ug/ml to ng/ uL

Results:. The concentration of our purified plasmid stock was displayed as 9.98 ug/mL/

$$\frac{9.98\ ug}{\cancel{mL}} \times \frac{\cancel{1000}\ ng}{\cancel{1\ ug}} \times \frac{\cancel{1\ mL}}{\cancel{1000}\ uL} = \frac{9.98\ ng}{uL}$$

Conclusion:.

/*----------*/

Page: 18

Date:. 4-23-2014

Title:. Gel Purification of merAB fragment

Purpose:. To purify the piece of DNA containing merAB from the gel area carrying it. Gel pieces 1 and 3 were used.

Methods:.

- Ad 1.2 mL of L3 buffer to gel slice
- Place in 55^0C incubator, invert tube every 3 minutes. Incubate until gel melts
- After gel has melted, incubate for 5 more minutes.
- Purify using a centrifuge y following the steps outlined in the PureLink Quick gel extraction kit.
    1. Pipet 800 uL of dissolved gel onto an extraction column inside a wash tube
    2. Centrifuge at 12000 x g for 1 minute
    3. Discard flow through, place the column into the wash tube
    4. Repeat steps 1-3 with the remaining volume of dissolved gel
    5. Follow steps 3-7 in the kit protocol. Repeat for Gel 3.
    6. Quantify DNA using the protocol on page 17

Results:. merAB fragment from 10um primer PCR 21.1 ug/mL
merAB fragment from 100 uM primer = 3 mg/uL

Conclusion:.

/*----------*/
Page: 19

Date:. 4/23/14

Title:. Creating -80 stock cultures of pUQ950 *E. coli* strain containing the pBBR-BB plasmid

Purpose:. To make a main stock of the pBBR-BB plasmid

Methods:.
Add 30 ul of 50% Glycerol
700 uL of overnight culture
Mix and remove air bubbles
Store at -80^0C
Saved as JG2966

Results:.

Conclusion:. Stored at -80^{0}C and saved as JG2966

/*----------*/
Page: 20

Date:. 4-23-14

Title:.Purification of pBBR-BB plasmid from pUQ950 strain

Purpose:. Purification of pBBR-BB plasmid for storage at -20^{0}C using the Quick Plasmid Miniprep kit

Methods:.
Use the PureLink Quick Plasmid Miniprep kit to purify the plasmids

- Retrieve the four tubes of overnight cultures of transformed pUQ950 with pBBR-BB (prepped 4/22/14 by transferring 4 colonies from 1:10 dilution plate to LB-KM medium; see page 14).
- Complete steps base on protocol on page 8.
- Quantify the plasmids in each tube individually using the protocol on page 17 and the Qubit Quant-It Broad Range kit.

Results:.The concentrations of the four tubes of purified pBBR-BB:

1. 9.04 ng/uL
2. 16.3 ng/uL
3. 21.9 ng/uL
4. 18.1 ng/uL

Conclusion:.Purified pBBR-BB plasmid tubes 1-4 were stored in the iGEM 2014 freezer box in the walk-in freezer 361 at -20^{0}C

/*----------*/
Page: 21

Date:. 4-24-2014

Title:.PCR amplification of mer operon

Purpose:. Using merR fwd primer, and the DNA produce of PCR 1 (see page 15) to amplify the mer operon region in pDU1358 (from page 17) with Biobrick specific restriction sites flanking the mer operon product

Methods:.

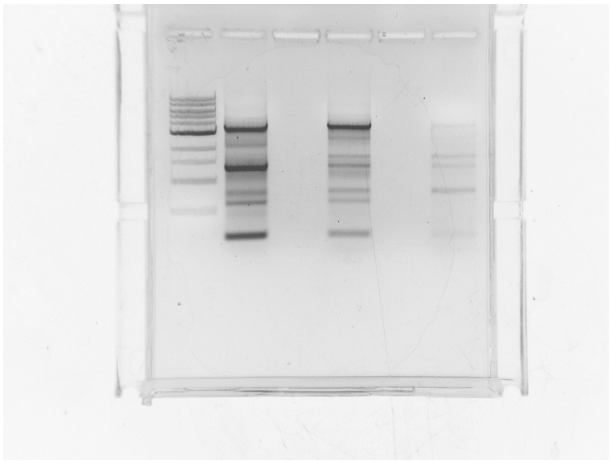
- Thaw pDU1358 purified plasmid 1 on ice
- Green mix 2x
- merR fwd primer (10um)
- merAB (21 ng/uL) DNA product from 10 uM primer reaction & gel piece 1 extraction
- mix 3 pcr tubes as follows:

	H	M	L
Green mix (uL)	25.0	25.0	25.0
10uM Fwd (uL)	2.5	2.5	2.5
prod primer (uL)	21.0	10.0	2.5
pDU1358 (uL)	1.0	1.0	1.0
Water (uL)	-	11.0	8.5
HF poly (pfu Ultra)(uL)	0.5	0.5	0.5
Total (uL)	50	50	40

- Run PCR at

1	2	3	74	5	6	7
95^0C	95^0C	62^0C	72^0C	steps 2-4	72^0C	10^0C
2:00	0:30	0:30	3:30	28x	5:00	oo
Denature		Anneal	Extend		Final Extension	

- a gel was prepared following procedure on p 22 and poured. Gel was loaded with 46 (M) and 47 (M)
- PCR tubes were place in far left “BioRad” thermocycler running “YUNICA” setting



Results:.

Conclusion:.

/*----------*/

Page: 22

Date:.4/24/14

Title:.Making Agarose and Ethidium Bromide Gel

Purpose:. To make a gel and prepare for electrophoresis

Methods:.

- Measure 400 mL of 1x TAE buffer
- Add 4g of Le agarose to make 1% agarose gel
- Heat for 4 minutes
- Take, out stir once gel starts boiling
- If Not completely molten, reheat
- Add 1 drop of EtBr
- Let cool, store in 55^0C

Results:.

Conclusion:.

/*----------*/

Page: 23

Date:. 4/25/14

Title:.To purify the piece of merTPAB fragment

Purpose:. To purify the piece of merTPAB DNA from the gel carrying it.

Methods:.
Follow the protocol from Invitrogen using the PureLink Quick Gel Extraction Kit and a centrifuge.

- *Add the appropriate amount of Gel Soluvilization buffer (L#) to gel fragment.
- For ~200 mg of gel with $\leq$ 2% agarose, 600 uL of L3 buffer was used. Follow the remaining steps to purify the DNA using the protocol for purifying DNA using a centrifuge found in the kit and on page 18.
- *Note: In the step 6, 100uL of elution buffer (E5) was added to the column in error

Results:.The concentration for the purified merTPAB DNA s 4 ng/uL
100 uL of DNA were obtained 400 ng of DNA in elution buffer.

Conclusion:.

/*----------*/
Page: 24

Date:. 4/28/14

Title:. Preparation of mer insert restriction digest

Purpose:. To digest the mer insert and create sticky ed that can be ligated into the pBBR-BB plasmid

Methods:.
Mix
1 ul XbaI enzyme
1 ul SpeI enzyme
6 ul NF H_2O
0.4 ug (in 100 ul) DNA
12 ul CutSmart NEBuffer (10x)

Tap and spin down. Then incubate for 1 hour at 37^0C .
Heat inactive enzymes for 10 minutes (typically 20) @ 70^0C,
Sit for 5 minutes to come back to room temperature and denature DNA to come back together follow procedure in quick PCR purification kit
480 ul of binding buffer 2 were added to 120uL of DNA and elution buffer (50 ul)

Results:. Digested merRTPAB fragment 8.47 ng/ul

Conclusion:.

/*------------------------------*/
Page: 25

Date:.

Title:.

Purpose:.

Methods:.
NEB>HOme> Technical References> Interactive Tools> Double Digest finder

Results:.
XbaI and SpeI both have 100% activity in CutSmart

Conclusion:.

/*------------------------------*/
Page: 26

Date:. 4/29/14

Title:. Restriction Digest of pBBR-BB

Purpose:. To digest the backbone pBBR-BB with sticky ends created for the mer operon to be ligated into.

Methods:.
Fill a bucket of ice and retrieve the purified pBBR-BB from the -20^{0}C. pBBR-BB purified tube 3 of concentration 21.9 ng/ul was used
Follow the recipe from New England Biolabs (see iGEM protocols binder.)
Mix
1.0 ul XbaI
1.0 ul SpeI
75.0 ul DNA
9.0 ul 10x NEBuffer
4.0 ul NF water
90.0 ul Total Reaction Volume

Add all the ingredients to a single tube, and add restriction enzymes last.
Tap to mix, spin down ~5-7 seconds in microcentrifuge.
Incubate at 37^{0}C for one hour

Dephosphorylate the 5' end of the digested plasmids using the protocol from NEB (see iGEM protocols binder)
Incubate for 80 minutes at 37^0C
    10ul 10x Antarctic phosphatase reaction buffer and
    1ul Antarctic phosphatase were added
After incubation heat inactivate the enzymes by incubating the tube in a hot water bath (70^0C) for 5-10 minutes
Let the tube sit at room temperature for 5 minutes to allow denatured DNA to come back together.
Load all the of the DNA digested into a gel and run it for 40 minutes. Use on undigested sample (~10) of plasmid DNA as a control
Photograph the gel results and print the image. Cut out the digested backbone bands and store gel pieces in the iGEM 2014 box in the -20^0C freezer.

Results:.

Conclusion:.

/*----------*/
Page: 27

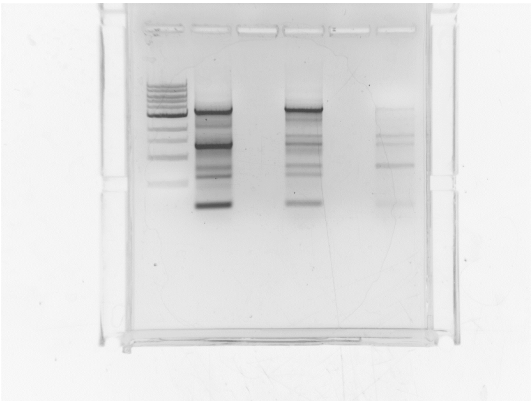
Date:.

Title:. (Continuation from pg 26)

Purpose:.

Methods:.

Results:.



The gel slice of DNA were gel purified and quantified on 4/30/14
Digested pBBR-BB concentration = 4 ng/ul

Conclusion:.

/*----------*/
Page: 28

Date:. 4/30/14

Title:. Ligation of Digested mer operon fragment into digested pBBR-BB backbone

Purpose:. To ligate the mer operon insert into the pBBR-BB backbone

Methods:.Determine the volume of insert DNA and the volume of plasmid DNA to be ligated. Use the protocol called “Ligation Protocol with T4 DNA Ligase” in the iGEM protocol binder.

Use 10 ng of plasmid DNA per kb: ~ 5kb plasmid → ligation 50 ng
Use insert vector in quantity ratios of 7:1, 5:1, and 3:1. Also set up a negative control with plasmid DNA only (pBBR-BB w/o insert).

$$50\ \text{ng}\ \cancel{\text{plasmid}} \times \frac{1.3\ \cancel{\text{kb}}\ \text{mer insert}}{4.5\ \cancel{\text{kb}}\ \cancel{\text{plasmid}}} \times \frac{3}{1} = 43.3\ \text{ng insert}$$

$$\frac{5}{1} = 72.2\ \text{ng insert}$$

$$\frac{7}{1} = 101.1\ \text{ng insert}$$

$$50\ \cancel{\text{ng}} \times \frac{1\text{ul}}{4\ \cancel{\text{ng}}} = 12.5\ \text{ul plasmid}$$

$$43.3\ \cancel{\text{ng}} \times \frac{1\text{ul}}{8.47\ \cancel{\text{ng}}} = 5.1\ \text{ul plasmid}$$

$$72.2\ \cancel{\text{ng}} \times \frac{1\text{ul}}{8.47\ \cancel{\text{ng}}} = 8.5\ \text{ul plasmid}$$

$$101.1\ \cancel{\text{ng}} \times \frac{1\text{ul}}{8.47\ \cancel{\text{ng}}} = 11.9\ \text{ul plasmid}$$

The following volumes were used for the ligation reaction (ul)

	3:1	5:1	negative control
plasmid	12.5	12.5	12.5
insert	5.1	8.5	0

10x buffer	2.0	2.5	2.0
NF Water	0	1	5
Ligase	0.5	0.5	0.5

Ligation tubes were placed in the 16^0C incubator and incubated overnight

Results:.

Conclusion:.

/*----------*/
Page: 29

Date:. 5/1/2014

Title:. Transformation of UQ950 *E. coli* with pBBR-BB plasmid containing merRTPAB insert

Purpose:. To allow competent UQ950 *E. coli* cells to take up the ligated plasmid products and express kanamycin resistance-related proteins on an LB + Kan plate

Methods:. Obtain 3:1, 5:1, and -control tubes from 16^0C incubator.
Place on ice
Obtain UQ950 competent cells from -80^0C incubator (3)
Place on ice, allow to thaw
Add 10 ul of each of the plasmid DNA concentrations to their respective UQ950 tube. Tap tubes.
Keep on ice 30 min
Set water bath to 42^0C
Heat Shock for 1 minute, ice for 5 minutes
Incubate (shaking for 1 hour at 37^0C; not for growth, time to make KanR gene product that confer resistance
Preheat 5 plates at 37^0C
Plate 75ul of each sample on respective plate (less). Centrifuge at 8,000 x g for 2 mins
Pipet 75 ul of each sample, discard the supernatant.
Resuspend pellet w/ 75 ul sample
Plate on rspective plates (more concentrated).

Plate	5:1 less	3:1 less	-control less	5:1 more	3:1 more	-control more
# of colonies	20	9	0	2	1	0

15h: No growth on -control.

Results:.

Conclusion:.

/*----------*/
Page: 30

Date:. PCR screen of UQ950 *E. coli* containing pBBR-BB containing merRTPAB insert

Title:. To screen for colonies carrying desired plasmid using PCR Amplification w/ pBB in F and pBBinR primers

Purpose:. Prepare 450 ul PCR mix as follows:

12.5 ul Green Mix		225 ul
0.5 ul Fwd primer (pBBinF)	x 17 +1 =	9 ul
0.5 ul Rev primer (pBBinR)		9 ul
11.5 ul NF water		207 ul
		450 ul

Aliquot 25 ul into each of 18 PCR tubes
Only using 17 tubes
Use pipette tip to pick a colony
Swirl in PCR mix aliquot
transfer to new Lb + Kan plates
Pipette 0.5 ul of pBBR-BB (2) purified plasmid into tube 17 to as as - control

5:1 and 3:1 plates placed in fridge, undergrad shelf mer colony screen places in 37^0C incubator for 72 hours

PCR run at

1	2	3	4	5	6	7
95^0C	95^0C	55^0C	72^0C	steps 2-4	72^0C	10^0C
2:00	0:30	0:30		30x	5:00	oo

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 32

Date:. 5/6/14

Title:. Preparing Overnight Cultures of pBBR-BB::merRTPAB

Purpose:. To prepare overnight cultures of UQ950 *E.coli* containing the pBBR-BB plasmid with mer operon insert.

Methods:. The protocol for making overnight cultures for strains found on pg 5-6 was used to prepare cultures for colonies picked off of streak plates of colonies 3, 4, 5, 15, and 16. (According to the gel results, these colonies appeared to contain pBBR-BB ::merRTPAB>)

The 5 tubes (labeled 3, 4, 5, 15, and 16) were placed in the 37^0C incubator while shaking, overnight.

Results:.

Conclusion:.

/*----------*/
Page: 33

Date:. 5/7/14

Title:.Purification of pBBR-BB::merRTPAB from *E. coli* strain

Purpose:.To purify the biobrick plasmid pBBR-BB::merRTPAB containing our mer operon insert to store at -20^0C

Methods:. Use the PureLink Quick Plasmid Miniprep kit from Invitrogen to purify the plasmids. Follow the protocol for purification using a centrifuge which is found inside the kit and the protocol on page 3 of this notebook.

Following purification, use the Qubit Quant-It Broad Rage kit and follow the the protocol on page 17 of the notebook to quantify the purified pBBR-BB::merRTPAB stocks. Following quantification; the tubes were stored in the -20^0C freezer in the iGEM 2014 freezer box.

Results:.

pBBR-BB::merRTPAB tube #	Concentration (ng/ul)
4	26.0
6	55.8
15	21.6
16	22.0
3	17.7

Conclusion:.

/*----------*/
Page: 34

Date:. 5/20/14

Title:. Restriction Digest of pBBR-BB::merRTPAB using XbaI and SpeI

Purpose:.To determine which colonies of transformed UQ950 *E. coli* cells contain pBBR-BB::merRTPAB with the mer operon insert in the forward orientation (merRTPAB genes flacked by XbaI and SpeI sites. merBAPTR would be flanked by scars and would not be digested.)

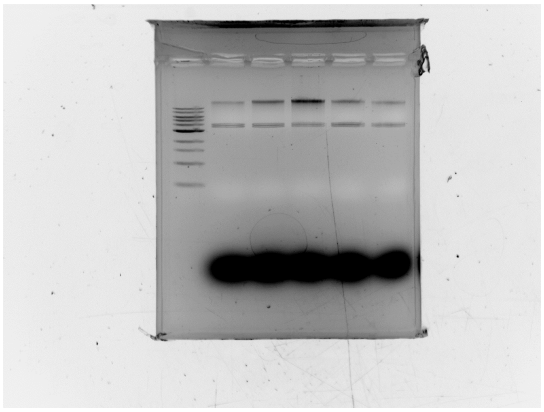
Methods:.

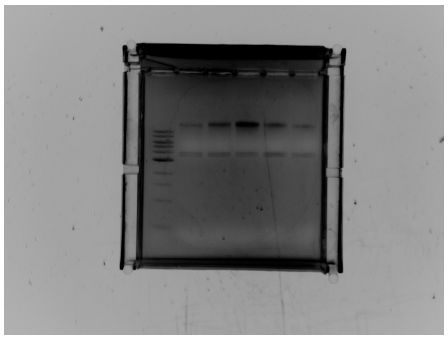
- Fill a bucket of ice and retrieve the purified pBBR-BB::merRTAB 3,4,6, 15,16 tubes for testing from the -20^{0}C freezer.)
- Follow the protocol for Optimizing Restriction Endonucleases Reactions from NEB (in the iGEM protocols binder).
- The following digests were prepared

Tube #	3	4	6	15	16
SpeI (ul)	1	1	1	1	1
XbaI (ul)	1	1	1	1	1
DNA (ul)	6	5	5	5	5

10x NEBuffer (ul)	1	1	1	1	1
NF Water (ul)	1	2	2	2	2
Total (ul)	10	10	10	10	10

Results:.





Conclusion:.

/*----------*/

Page: 35*blank

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

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Page: 36

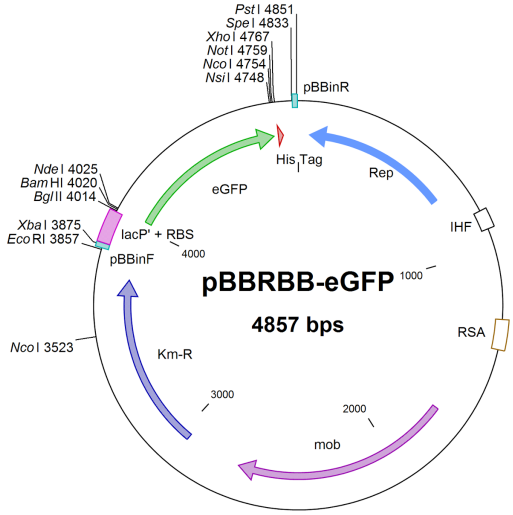
Date:.

Title:.

Purpose:.

Methods:.

Results:. NEB Cutter restriction map of pBBRBB-eGFP. Image not shown, map and sequence available from https://www.addgene.org/32549/:



Conclusion:.

/*----------*/
Page: 37

Date:. 5/23/14

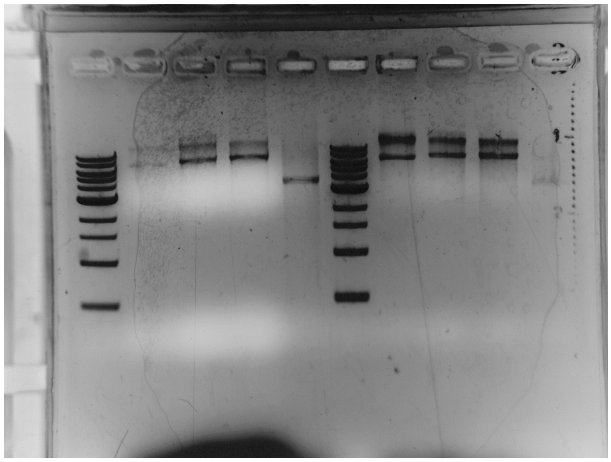
Title:.

Purpose:. To perform restriction digest and determine the correct orientation of the mer operon insert within the pBBR-BB plasmid, determine the size of the plasmid, the size of the insert, and where the plasmid runs in the gel if uncut

Methods:. Set up 4 restriction digests for plasmids 3 & 4 pBBR-BB::merRTPAB as follows

	DNA (ul)	NEBuffer (ul)	NF water (ul)	Re#1 (ul)	RE #2 (ul)
3.1	5	1	4	0	0
3.2	5	1	3.5	0.5 (BsaI)	0
3.3	5	1	3	0.5 (BsaI)	0.5 (SacI)
3.4	5	1	3	0.5 (EcoRI)	0.5 (PstI)
4.1	5	1	4	0	0
4.2	5	1	3.5	0.5 (BsaI)	0
4.3	5	1	3	0.5 (BsaI)	0.5 (SacI)
4.4	5	1	3	0.5 (EcoRI)	0.5 (PstI)

Results:.



Conclusion:.

/*----------*/

Page: 38

Date:. 5/23/14

Title:.

Purpose:.

Methods:. Papers

Results:.

Conclusion:.

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Page: 39*blank

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.
/*----------*/
Page: 40

Date:. 5/24/14

Title:.

Purpose:. To perform a second restriction digest to determine plasmid size, number of EcoRI cut sites, and mer insert orientation

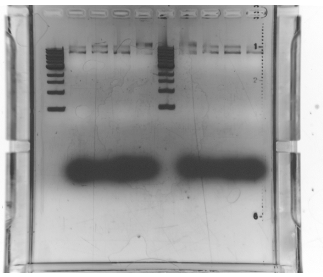
Methods:. Set up restriction digests as follows

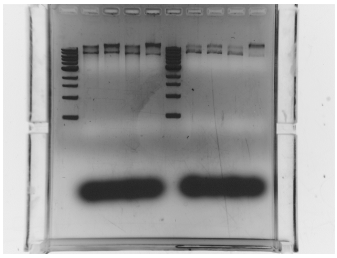
	DNA (ul)	BSA (ul)	NEBuffer (ul)	NF Water (ul)	RE#1 (ul)	RE#2 (ul)
6.1	5	0.5	5	39.0	0.5 (EcoRI)	0
6.2	5	0.5	5	39.0	0	0.5 (NheI)

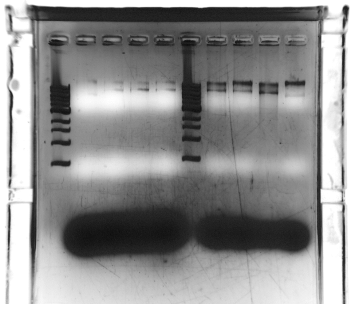
6.3	5	0.5	5	38.5	0.5 (EcoRI)	0.5 (NheI)
6.4	5	0.5	5	39.5	0	0
15.1	5	0.5	5	39.0	0.5 (EcoRI)	0
15.2	5	0.5	5	39.0	0	0.5 (NheI)
15.3	5	0.5	5	38.5	0.5 (EcoRI)	0.5 (NheI)
15.4	5	0.5	5	39.5	0	0
16.1	5	0.5	5	39	0.5 (EcoRI)	0
16.2	5	0.5	5	39	0	0.5 (NheI)
16.3	5	0.5	5	38.5	0.5 (EcoRI)	0.5 (NheI)
16.4	5	0.5	5	39.5	0	0

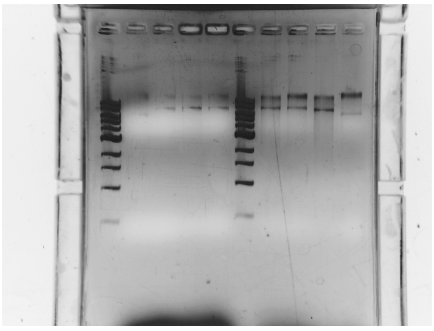
6.1 - 6.4 incubated @ 37^0C at 1:50 p.m.
15.1 - 15.4 incubated @ 37^0C at 2:10 p.m.
16.1 - 16.4 incubated @ 37^0C at 2:30 p.m.

Results:.









Conclusion:.

/*----------*/

Page: 41

Date: 5/30/2014

Title: Isolation of pBBRBB::merTPAB

Purpose: To isolate pBBRBB::merTPAB from overnight cultures of candidate colonies 3, 4, 6, 15, and 16. Samples will be used to confirm mer operon orientation by sequencing.

Methods: Followed PureLink miniprep protocol (page 8).

Results: Obtained 50 uL elute from each miniprep. Quantified via Qubit Quant-It Assay:

Colony	**Concentration (ng/uL)**
3	4.05
4	Out of range < 0.010 uG/uL
6	Out of range < 0.010 uG/uL
15	Out of range < 0.010 uG/uL
16	2.8

Conclusion: Stored elutions in 2014 iGEM -20oC freezer box. For next time, linearized with EcoRI to verify mutagenesis within *merA* and to check for presence of a greater than 10 kb band which has been seen in previous gels loaded with pBBRBB::merTPAB digests.

/*----------*/

Page: 42

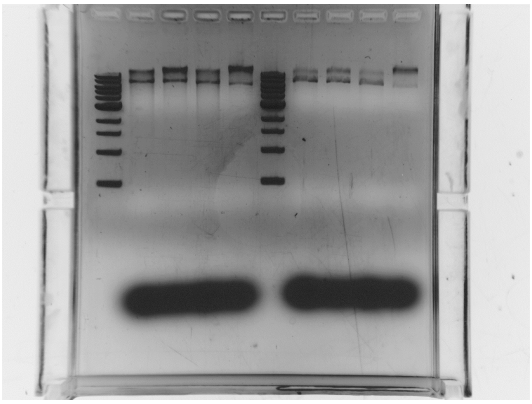
Date: 5/24/2014

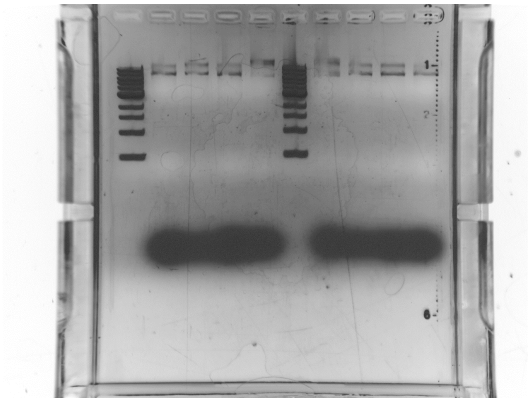
Title: N/A

Purpose: To run a gel on restriction digests' products and check band sizes. This will allow us to ascertain the size of the plasmid (linearized), orientation of insert, and where the uncut plasmid runs in the gel.

Methods: Followed gel protocol on page 16

Results and Conclusion:





For 6.1, 6.2, 15.1, 15.2, 16.1, 16.2, a band appeared at 7.4 kb, which correlates with the size of linearized pBBRBB lacking an insert + the mer insert. The uncut plasmid (6.4, 15.4, 16.4) ran slightly further and showed a slightly smaller sized-band, which could be due to supercoiling of the plasmids. 15.3, 16.3, and 6.3 ran at 6.9 kb as expected if the mer operon was inserted correctly and cut with EcoRI and NheI. The 500 bp band may not have been visible due to low DNA concentration.

/*----------*/

Page: 43* blank

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

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Page: 44*blank

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

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Page: 45

Date:.

Title:.

Purpose:.

Methods:. Papers

Results:.

Conclusion:.

/*----------*/
Page: 46

Date:. 6/2/14

Title:.

Purpose:. To streak LB+Kan plates with cells containing pDU1358, and cells containing pBBR-BB from the -80^{0}C sto. The pDU1358 containing cells will be used to clone individual parts, whereas pBBR-BB will be used as the vector in which the mer parts are inserted

Methods:.

- Obtain -80^{0}C stocks of
    - pDU1358 JG2954 strain
    - pBBR-BB JG2966
- Streak onto separate LB+Kan plates
- Place plates in 37^{0}C incubator

Results:.

Conclusion:.

/*----------*/
Page: 47

Date:. 6/2/14

Title:.

Purpose:.Create overnight liquid cultures using mer containing colonies: #3, 4, 6.

Methods:. Follow protocol from page 6.

Results:.

Conclusion:. Place in 37^{0}C agitating incubator

/*----------*/
Page: 47

Date:. 6/3/14

Title:.

Purpose:. To purify the pBBR-BB plasmids from colonies 3, 4, 6

Methods:.Follow protocol on page 8. Pre-heat the TE buffer, only use 40 ul for elution

Results:.
Colony 3 pBBR-BB mer plasmid DNA concentration = 59.5 ng/ul
Colony 4 pBBR-BB mer plasmid DNA concentration = 60.3 ng/ul
Colony 6 pBBR-BB mer plasmid DNA concentration = 48.9 ng/ul

Conclusion:.

/*----------*/
Page: 48

Date:. 6/3/14

Title:.

Purpose:. To create overnight cultures for pBBRBB::GFP colonies and pDU1358 colonies from plates made on 6/2 (page 46)

Methods:. Follow protocol on page 6.

Results:.

Conclusion:. Placed in 37^0C shaker

/*----------*/
Page: 49

Date: 6/4/14

Title: Purification of pDU1358 and pBBRBB

Purpose: To purify pDU1358 and pBBRBB from overnight cultures

Methods: Follow protocol on page 8 on two samples of pDU1358 and one of pBBRBB

Results: Obtained 45 uL elute from each miniprep. Quantified via Qubit Quant-It Assay:

Molarity (ug/ml)	Sample
2.48	pDU1358 trial 1
25.2	pBBRBB
5.75	pDU1358 trial 2

Conclusion: Plasmids have been purified to a high enough concentration to be used for amplification

/*--------------------------------*/
Page: 50

Date:. 6/4/14

Title:.

Purpose:. Purify pDU1358 and pBBRBB plasmids from overnight cultures

Methods:. Followed protocol from page 5
Mini prep 1-3 pDU1358 and 9-12 pBBRBB
Quantified DNA using Qubit fluorometer

Results:.
3 - 5.6 ug/ml
10 - 2.97 ug/ml
11-6.37 ug/ml

Conclusion:.

/*--------------------------------*/
Page: 51

Date:. 6/6/14

Title:.

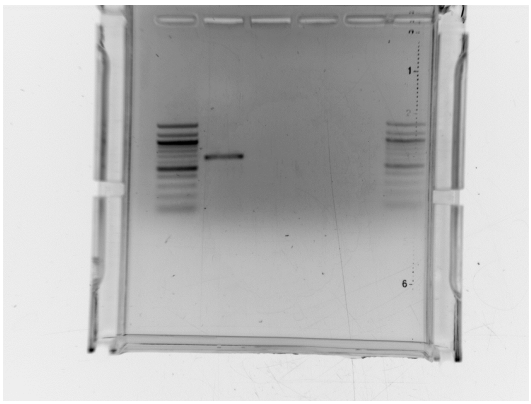
Purpose:. Parts cloning (merB)

Methods:.

1. Rehydrate merB_F and $ BB primers to 100 uM
2. Dilute the primers to 10 uM in separate 1.5 mL tubes
3. Set up the Rxn in the PCR tube.
   1 ul pDU1358 (5.75ng/ml)
   0.5 ul merB R BB @ 10 uM
   0.5 ul merB F BB @ 10 uM
   25 ul GoTaq mix Green mix
   22.5 ul NF Water
   0.5 ul pFU-Ultra DNA polymerase
4. Run PCR at

	1	2	3	4	5	6	7

Temp (^{0}C)	95	95	57	72	x28	72	10
Time (min)	2:00	0:30	0:30	1:30	2-4	5:00	oo

Results:. 

Conclusion:.

/*----------*/
Page: 52*paper

Date:. 6/5/14

Title:.

Purpose:. To sequence the mer insert in the pBBR-BB vector plasmid, ensure its identity as the merRTPAB operon, and that the EcoRI site in merA was mutagenized and no longer encodes a restriction site

Methods:. Create a 100 uM stock of sequencing primers using NF water
create a 10 uM stock of sequencing primers labelled as
pBBRBB_seq _F ----> BAS1
pBBRBB_seq_R ----> BAS2
mer_seq1_F
mer_seq2_F
mer_seq3_F
mer_seq4_F
mer_seq5_F
mer_seq6_F
mer_seq7_F
mer_seq8_F

For each sequencing reaction,

3 ul Template DNA (183 ng)
1 ul Primer (10uM)
8 ul NF Water
12 ul total reaction

Results:.

Conclusion:. Submitted BAS1 and BAS2 reaction tubes for sequencing

/*----------*/
Page: 53

Date:. 6/9/14

Title:.

Purpose:. Parts cloning (merB) repeat

Methods:.PCR product purification failed, repeat procedure on page 51

Results:.

Conclusion:.

/*----------*/
Page: 54

Date:. 6/9/14

Title:.

Purpose:.Backbone digestion for shipping parts submission

Methods:.

1. Make restriction enzyme master mix
   2.5 ul Cutsmart Buffer
   0.5 ul XbaI
   0.5 ul SpeI
   0.5 ul DpnI

21 ul NF water
25 ul tota

2. Digest backbone plasmid pSB1C3
    a. Add 4 ul of linearized plasmid backbone (25 ng/ul)
    b. Add 4 ul of enzyme master mix
3. Digest at 37^0C for 30 minutes
4. Heat kill at 80^0C for 20 minutes

Results:.

Conclusion:.

/*----------*/
Page: 55

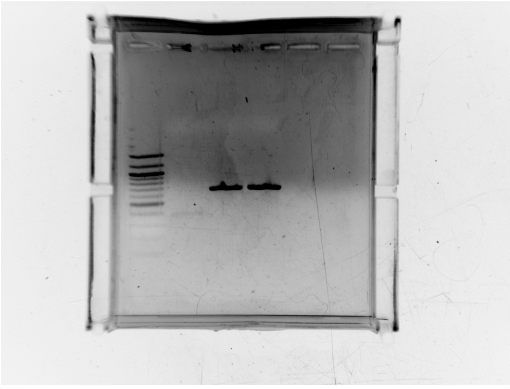
Date: 6/9/14

Title: Amplification and Verification of MerT using PCR and gel electrophoresis respectively

Purpose: To amplify MerT using PCR and verify it by running a gel

Methods: See page 51

Results: Quantified using Qubit Assay: 2.80 ug/ml



Conclusion: PCR was redone to low molarity and ready to have another gel run of it

/*----------*/
Page: 56

Date:. 6/9/14

Title:.

Purpose:. Verify that merB ad merT have been successfully amplified by PCR and column purified

Methods:.
Gel Electrophoresis

1. Runa gel electrophoresis on the merB d merT sample along with 100 base pair DNA ladder
2. Add 1x TAE buffer to tray
3. Pour agarose into the tray and allow to solidify for 20 minutes
4. Pipette samples into gel and run at 120V for 45 minutes

Column Purification

1. Add 4 volumes of B2 binding buffer to 1 volume of merB sample (40ul)
2. Load into a collection tube and spin >10,000 for 1 min
3. Wsh with 650 ul of wash (W1) buffer
4. Elute with 40 ul of elution buffer

Results:. Need picture

Conclusion:.

/*----------*/
Page: 57

Date:. 6/9/14

Title:.

Purpose:.To amplify merP

Methods:.

1. Rehydrate plasmids merP_RBB and merP_FBB and make stocks of 10 uM
2. Amplify merP from pDU1358 by the PCR procedure described in page 51.

Results:.need picture

Conclusion:.

/*----------*/
Page: 58

Date:. 6/9/14

Title:.

Purpose:. Double digest in order to produce merB insert with sticky ends

Methods:.
1.0 ul XbaI
1.0 ul XpeI
10.0 ul NeBuffer (Cutsmart)
38.0 ul DNA
50 ul total

Incubate for an hour

Results:.

Conclusion:.

/*----------*/
Page: 59

Date: 6/10/14

Title: Re Attempt of verification of MerT

Purpose: To run another gel to verify MerT, to purify it, and ultimately obtain a sample with a high enough concentration to perform a double digest.

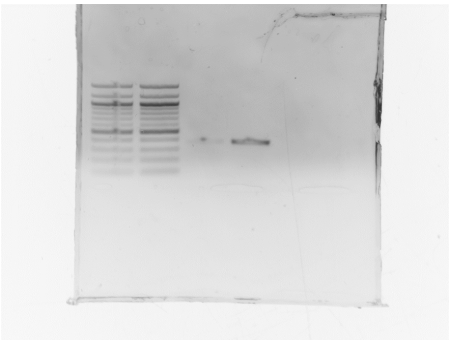
Methods:
Run gel electrophoresis with 100 bp ladder

1. Add 1X TAE buffer to chamber
2. Pour 1% agarose and give 20 min to solidify
3. Pipette 10ul of ladder and 5 ul of pcr merT into gel
4. Run Gel @ 120V for 45 min

Purification

1. Add 4 vol (160 ul) of B2 binding buffer to remaining PCR product (40ul) of merT
2. Load into collection tube and spin >10,000 g for 1 min
3. Wash with 650 ul of Wash buffer, spin >10,000 for 1 min, and spin again @ max speed for 2 min.
4. Add 40 ul of Elution buffer, incubate for 1 min, and centrifuge for 2 min @ max speed

Results: Obtained 40 uL elute of merT. Quantified via Qubit Quant-It Assay: 9.46 ul/ml



Conclusion: Results were still too low. Reattempt PCR.

/*----------*/
Page: 60

Date:. 6/10/14

Title:.

Purpose:. Amplify merA and make master plate for shipping vector

Methods:.
Amplification
For amplification see page 51
Transforming shipping vector (RFP construct BBa_Jo4450, 50 pg/mL) into UQ950

- Obtain UQ950 competent cells from -80^{0}C freezer
- Allow to thaw and add 1 ul of vector
- Incubate on ice for ~20 minutes
- Heat Shock for 1 min at 45^{0}C
- Add 1 ml LB media and leave on ice for 5 min
- Place in 37^{0}C shaking incubator for 1 hr
- Centrifuge the tube at max speed for 30 seconds
- Resuspend in 100 ul solution and plate on chloramphenicol plates
- Leave in 37^{0}C incubator overnight

Results:. No Growth

Conclusion:.

/*----------*/
Page: 61

Date: 6/10/14

Title: Amplifying merR

Purpose: to amplify merR using PCR for transformation into the shipping vector

Methods:

1. Rehydrate merR_R and merR_F primers to 100mM
2. Dilute to 10 mM in separate tubes by adding 10 uL of 100mN stock to 90 uL of ddH2O
3. Set up PCR reaction as follows

Phusion Buffer	10uL
merR_R	2.5uL
merR_F	2.5uL
Template (pDU1358)	0.5uL
dNTPs	1uL
Phusion Polymerase	0.5uL
ddH2O	33uL
Total Rxn Volume	50uL

4. Run PCR under following conditions, running steps 2-5 for 30 cycles

	1	2	3	4	5	6
temp. (degrees C)	98	98	58	72	72	4
time (seconds)	30	15	30	30	300	infinite hold

/*----------*/

Page: 62

Date:. 6/10/14

Title:.Double Digest of merT and shipping vector pBBRBB from XBaI to SpeI

Purpose:. To digest the mer operon component merT with XbaI and SpeI and pBBRBB XbaI-->SpeI in order to prepare merT to be ligated into the shipping vector as a part for submission.

Methods:. Follow the protocol Optimizing restriction endonuclease reactions from NE

Results:.

Conclusion:.

/*----------*/
Page: 63

Date: 6/10/14

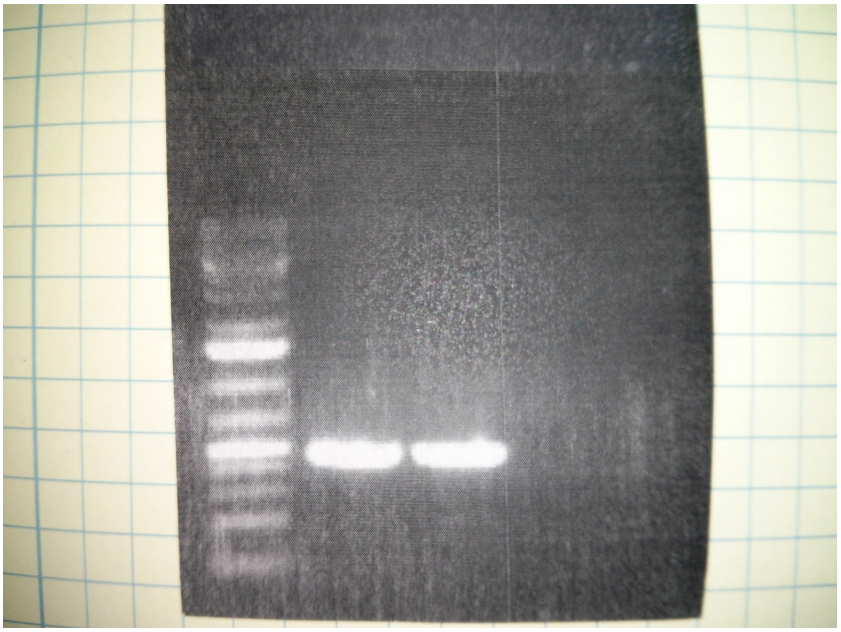
Title: Verifying merR PCR

Purpose: to verify amplification of merR and purify from gel and to check concentration of DNA after gel purification.

Methods:

1) Run sample on 1% agarose gel
2) Extract from gel according to protocol from Invitrogen kit
3) Find concentration using Qubit

Results:



DNA concentration: 19.5 ng/uL

Conclusion: The appropriate part was amplified and is ready for digestion.

/*----------*/
Page: 64*paper

Date:. 6/10/14

Title:. Digestion and Ligation Of mer P

Purpose:.To digest merP with XbaI and SpeI for its ligation into the shipping vector

Methods:.

1. The PCR product was purified using tea PCR purification kit
2. The product was then digested following the procedure on page 58 incubating the sample at 37^0C.
3. The digestion product was purified with a PCR purification kit
4. The Ligation could not be performed due to low concentration of the plasmid and insert

Results:.
insert 3.93 ng/ ml
plasmid (pSB1C3) 0.598 ng/ ml

Conclusion:.

/*----------*/
Page: 65

Date:. 6/10/14

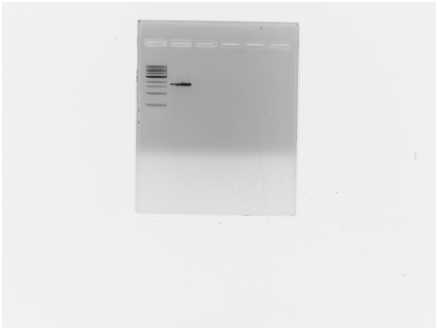
Title:.PCR purification and digest of merA

Purpose:. Purify PCR amplification of merA gen, digestion with XbaI and SpeI.

Methods:.

1. PCR purification kit used to purify product
2. Product from step 1 digested (procedure pg 58)
3. Digestion product purified using PCR purification kit
4. Dephosphorylation using AnP

Results:.
PCR product = 43 ug/mL



Conclusion:.

/*----------*/
Page: 66

Date:. 6/10/14

Title:. Ligation of merT into pSB1C3

Purpose:. To ligate merT (X/S) into prepared pSB1C3 (X/S)

Methods:. Using the ligation protocol with T4 DNA ligase from NEB (in the iGEM 2014 protocol binder) merT was ligated into the XbaI and SpeI site of pSB1C3, the shipping vector.
pSB1C3 - 2070 kb ---> 2.1 kb = use 21 ng plasmid. Rounded up to 25 ng
(pSB1C3 X/S of concentration 0.598 ng/ul was used)

25 ~~ng of~~ plasmid x $\frac{1\ ul}{0.598\ ng}$ = 41.8 ul of plasmid

25 ng ~~plasmid~~ x $\frac{0.35\ kb\ insert}{2.1\ kb\ plasmid}$ x 3 = 12.5 ng insert needed

$\frac{1\ ul\ merT}{1.08\ ng\ merT}$ x 12.5 ~~ng~~ insert = 11.57 ul merT

The following recipe was prepared.
41.8 ul plasmid
11.57 ul merT
6 ul T4 Ligase buffer (10x)
0.5 ul T4 DNA Ligase
0.1 ul NF Water

Results:.

Conclusion:. The ligation tube was labeled pSB1C3::merT and stored at 20^0C overnight (stored at 5 p.m.)

/*----------*/

Page: 67

Date: 6/11/14

Title: Re-amplification of merT and purification

Purpose: To reamplify merT and purify it for a higher concentration

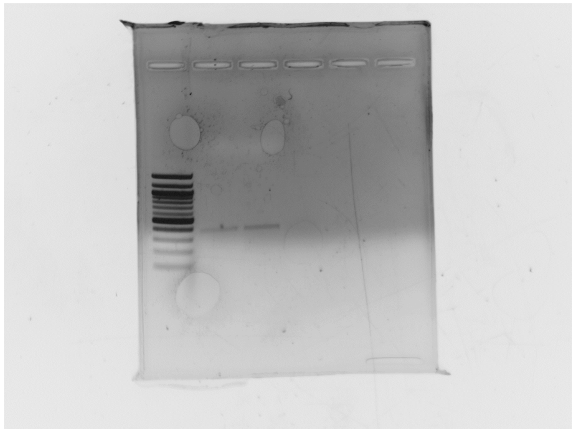
Methods:

Run PCR reaction in PCR tube

1ul pDU1358 (5.75 ul/ml)
0.5ul merT forward primer (10nM)
0.5ul merT reverse primer (10nM)
25ul GoTaq polymerase mix (2x)
22.5ul NF H_2O
0.5ul NF DNA polymerase (pFU ultra)

Temp (^{0}C)	95	95	57	72	repeat steps	72	10
Time (min)	2:00	0:30	0:30	1:30	2 -4 28 times	5:00	oo

Perform gel electrophoresis same as pg 59 except for only 35 min
Purification same as pg 59 except 140 ul of buffer (B2) and 35 ul of DNA
Results: Obtained 35ul elute of merT. Quantified via Qubit Quant-It Assay: 0.946 ug/ml



Conclusion: Re attempt PCR again

/*----------*/

Page: 68

Date:6/11/14

Title: Digestion of merR PCR product

Purpose: to digest merR PCR product from 6/10 XbaI/SpeI so that it can be ligated into a pSB1C3 backbone.

Methods: Follow protocol Optimizing Restriction Endonuclease Reactions from NEB (in iGEM 2014 protocol binder)

XbaI	0.5uL
SpeI	0.5uL
DNA	40uL
10X NEB buffer (cutsmart)	5uL
ddH2O	4uL
Total Rxn Volume	50uL

Combine and incubate 1 hour at 37^0C

/*----------*/
Page: 69

Date:. 6/11/2014

Title:.

Purpose:. To prepare a stock of pDU1358 from overnight cultures prepared on 6/10/14

Methods:. Followed PureLink miniprep protocol (pg. 8)
Combined 650 ul aliquots into final volume of 300 ul
Quantified via nanodrop

Results:. 300 ul of 86.3 ng/ul pDU1358

Conclusion:. Stored sample in iGEM freezer box

/*----------*/
Page: 70

Date:. 6/11/14

Title:.

Purpose:.Retransforming shipping vector into JM109 and ligating merA into pSB1C3

Methods:.
I retransformed the BBa?_J04450 (50 pg/ ul) in JM109

- Competent cells were thawed on ice
- added 5 ul o plasmid to the cells, incubate 10 minutes on ice
- Heat shock 1 min @ 42^0C, cool on ice 1 min.
- Add 1 ml LB and place in shaking incubator for 1 hour @ 37^0C
- Resuspend in 100 ul and plate on chloramphenicol plates
- Put plate in 37^0C incubator overnight.

Results:. Did not work

Conclusion:.

/*----------*/
Page: 70

Date:. 6/12/14

Title:.

Purpose:. To ligate merA into pSB1C3

Methods:.
Nanodrop data for insert

Concentration (ng/ ul)	260/280	260/230
11.9	1.94	.71

pSB1C3: 2070 bp (9.12 ug/ml)
merA: 1686 bp (11.9 ug/ml)

1686 ~~bp~~ merA x 20 ng ~~plasmid~~ = 16.29 ng merA
2070 ~~bp~~ ~~plasmid~~
16.29 ~~ng~~ merA = 1.4 ul merA for 1:1, 7.0 ul for 5:1, 4.1 ul for 3:1

11.9 ~~ng~~/ul

	5:1	3:1	-control
Plasmid (ul)	4.9	4.9	4.9
Insert (ul)	7	4.1	1
T4 Ligase Buffer 10x (ul)	2	2	2
T4 Ligase (ul)	0.5	0.5	0.5
Water	5.6	8.5	12.6

For a total of 20 ul reactions

Ligations were left at room temperature for one hour before transforming into UQ950 cells (page 74)

Results:.

Conclusion:.

/*----------*/
Page: 71

Date:. 6/11/14

Title:.

Purpose:. To create a stock of linearized pSB1C3 shipping vector for ligation reactions

Methods:.
Create enzyme master mix
- 21 ul NF water
- 2.5 ul CutSmart Buffer
- 0.5 ul XbaI
- 0.5 ul SpeI
- 0.5 ul DpnI

Add 4 ul of linearized plasmid backbone to 4 ul of reaction (100 ng in total)
Digest at 37^0C for 30 min
Heat kill at 80^0C for 20 mins
Add CIP (2 ul)
Digest at 37^0C for 1 hr

Use PCR purification protocol to clean up

Results:.

Conclusion:.

/*----------*/
Page: 72

Date:. 6/12/14

Title:.

Purpose:.To ligate merR into pSB1C3 backbone for subsequent transformation (both cut X/S)

Methods:.

- Purify digest w/ invitrogen kit --->in freezer box 2 (merR X/S 6/12 purified)
- Use 20 ng backbone (4.12 ng/ul)

1 ul x 20 ~~ng~~ = ~5 ul backbone
4.12 ~~ng~~

- Set up two ligation reactions (5:1)(3:1) Concentration of the insert = 13.8 ng/ul

1 ul x 100 ~~ng~~ = ~7.2 ul backbone
13.8 ~~ng~~
1 ul x 60 ~~ng~~ = ~4.2 ul backbone
13.8 ~~ng~~

- Set up reactions using the following recipes

	5:1	3:1
Backbone pSB1C3 (ul)	5	5
Insert merR (ul)	7.2	4.2
10x T4 DNA Ligase Buffer (ul)	2	2
T4 DNA Ligase (ul)	0.5	0.5
dd Water (ul)	5.3	8.3

Total for both rxn is 20 ul

- Incubate at room temperature for 1 hr
- Run on gel to confirm success of ligation.

Results:. See picture on pg 76 for results - inconclusive.

Conclusion:.

/*----------*/
Page: 73

Date:. 6/12/14

Title:.

Purpose:.To transform *E. coli* UQ950 with SB1C3 merR ligation mixture

Methods:. See page 29
Modifications

- Use LB + cm plates
- plate 75 ul from each rati
- spin down, remove 75 ul, Dump supernatant. Resuspend in 75 ul
- Plate 75 ul of new [] onto plates

Incubate overnight @ 37^0C.

Results:. No growth on any plates Reattempt with double digest

Conclusion:.

/*----------*/
Page: 74

Date:. 6/12/14

Title:.

Purpose:. To transform UQ950 cells with FRP BBa _J04450 for a third time since protocol on page 70 didn't work, and to transform UQ950 cells with pSB1C3 ad merA ligation from page 70

Methods:.
The protocol on page 29 as followed using 1 ul of RFP (pg/ml)

The protocol on page 29 was followed using 10 ul of the three ligation reactions from page 70. The 6 plates were placed in the incubator at 37^0C.

Results:.

Conclusion:.

/*----------*/

Page: 75

Date:. 6/12/14

Title:.

Purpose:. Ligate merB PCR product and pSB1C3 backbone (both X/S digested) and transformation of UQ950 *E. coli* with shipping vector pSB1C3 + merB

Methods:.

Ligations

1. Prepare 20 ul rxn (1:5 backbone = product)
   - 12.94 ul merB PCR product @ 7.73 ng/ul
   - 4.85 ul pSB1C3 @ 4.12 ng/ul
   - 2.00 ul 10x T4 DNA ligase buffer
   - 0.50 ul T4 DNA Ligase
   - 20.29 ul
2. Incubate @ room temperature for 1 hour

Transformation

1. Resuspend 10 ul of ligation product w/ 1 ml of RB
2. Follow protocol listed on page 29
3. Plate on designated LB + cam plate

Results:. Not sufficient enough of pSB1C3; no negative

Conclusion:.

/*----------*/

Page: 76

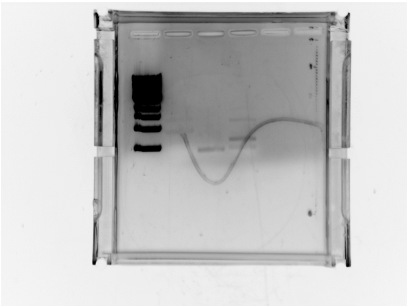
Date:. 6/12/14

Title:.

Purpose:. Gel electrophoresis to confirm ligated plasmid pSB1C3 mer B (1:5) and pSB1C3 merR (1:3)

Methods:.

1. Add 2 ul loading dye to 10 ul of ligation product
2. Load sample onto 1 % agarose gel. Run for 30 min @ 120V.



Results:.
Inconclusive at best. Empty merR 5:1 lane. Slightly longer than expected migration on merR 3:1 lane, possibly b/x circular plasmid. Multiple unexplained bands on merB 5:1 lane.

Conclusion:.

/*----------*/
Page: 77

Date:. 6/13/14

Title:.

Purpose:.Troubleshoot transformation methods of UQ950 with RFP BBa_J04450.

Methods:.Transformation UQ950 and C2566 with RFP and pACBB-GFP (also cm resistance).Using the protocol on page 29. Only competent cells with RFP were plated before and after centrifugation 1 ul of plasmid was used for 4 competent cell tubes. After incubation at 37^0C for 1 hour, the tubes were centrifuged at 4000 rpm before plating 75 ul on their respective plates. Competent cells with c2566 were only plated after centrifugation since they were merely a control.
A total of 12 plates were incubated at 37^0C overnight.

Results:.

LB+ cm	less RFP (UQ950)	more RFP (UQ950)	less RFP (C2566)	more RFP (C2566)	pACBB (UQ950)	pACBB (C2566)
Growth	-	-	-	-	+, lawn	-
LB	less RFP (UQ950)	more RFP (UQ950)	less RFP (C2566)	more RFP (C2566)	pACBB (UQ950)	pACBB (C2566)
Growth	+, lawn	+, lawn	+, lawn	+, lawn	+, lawn	+, lawn

Conclusion:.

/*----------*/
Page: 78

Date:. 6/14/14

Title:. Preparation of pBBRBB for cloning

Purpose:.To prepare pBBRBB BglI-->NotI for insertion of individual parts.

Methods:.The following digest was prepared in duplicate.

70 ul pBBRBB-eGFP
10 ul NEBuffer 3.1
2 ul BglI
2 ul NotI
16 ul Water

Incubate overnight at 37^0C
Gel extraction of linearized plasmid backbone.
Run digests (100 ul each) on 1% agarose gel with ethidium bromide. Purified DNA using Qiagen Quick gel Extraction Kit

Results:. Quantification was below threshold for Qubit. Will prepare a new digest.

Conclusion:.

/*----------*/
Page: 79

Date:. 6/14/14

Title:. Overnight cultures of pSB1C3 - Banana and pSB1C3 - CFP

Purpose:. To prepare shipping vector (pSB1C3) in high yield for downstream processes.

Methods:. Single transformants were transferred to 5 ml LB + cm (30 ng/ml) and grown overnight in 37^0C shaker.

Results:.Growth present in all tubes

Conclusion:. Will miniprep to isolate plasmid.

/*----------*/

Page: 80*pages

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 81

Date:. 6/16/14

Title:.

Purpose:.PCR for merB- parts amplification and characterization

Methods:.-

1. Obtain primers for parts amplification. (merB FBB & merBRBB ; 10 uM working stocks).
2. Make primer for characterization 100 uM; make working stocks of 10 uM (merB F. BglI & merB R. NotI)
3. Set up PCR rxn:
   1.0 ul pDU1358
   0.5 ul F. primer (10 uM)
   0.5 ul R. primer (10 uM)
   25 ul Go Taq (2x)
   22.5 ul NF water
   0.5 ul HF DNA polymerase (pFU ultra)
   50 ul
4. Run PCR using protocol on page 51

Results:.

Conclusion:.

/*----------*/
Page: 82

Date:. 6/16/14

Title:.

Purpose:. Re-digest merR w/ XbaI and SpeI for ligation w/ pSB1C3 backbone.

Methods:.
Follow the protocol optimizing restriction endonuclease reaction from NEB (in iGEM 2014 protocol binder).

*Limited amount of mR PCR product left. Adjust recipe to fit amount remaining.

0.5 ul XbaI
0.5 ul SpeI
10 ul DNA (merR)
2 ul 10x NeB buffer (cutsmart)
7 ul dd Water
20 ul total volume

Combine and incubated at 37^0C for 1.5 hours.
Purify w/ invitrogen kit.

Results:.

Conclusion:. Sample inbox 2, spot 35.

/*----------*/
Page: 83

Date: 6/16/14

Title: Amplify merT with BglI and NotI

Purpose: To amplify merT with BglI and NotI using PCR

Methods: See pg 67 using the correct combination of 10 nM primers

Results: MerT was amplified

Conclusion: Ready for amplification

/*----------*/

Page: 84

Date:. 6/16/14

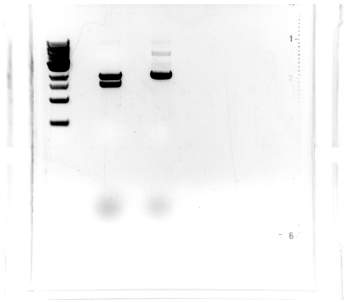
Title:.

Purpose:. Run gel to see if pSB1C3 (X/S) is successfully digested

Methods:.
*pSB1C3- banana digested for 11 hours

1. Make a 1% agarose gel w/ EtBr
2. Run samples as following 30 min at 120V

Lane 1: Ladder @ 1 kb. 5ul
Lane 3 digested pSB1C3-banana
Lane 5: undigested pSB1C3-banana

Results:. 

Conclusion:.

/*----------*/
Page: 85

Date:. 6/16/14

Title:. Making overnights of pSB1C3:;CFT, pBBRBB::GFP

Purpose:. To make overnight cultures of UQ960 *E. coli* containing pSB1C3::CFP shipping vector w/ insert, as well as UQ950 containing pBBRBB::GFP. These will be digested to obtain vectors for parts submission and characterization, respectively. Additionally, they will be used to create -80^{0}C stocks for the Gralnick lab. UQ950 containing pSB1C3::BBa_J45014 will also be used for the latter purpose.

Methods:. Follow protocol on page 6, using km for pBBRBB and cam for pSB1C3. Incubate (shaking at 37^{0}C overnight.

Results:.

Conclusion:.

/*----------*/
Page: 86

Date:. 6/16/14

Title:.

Purpose:.Gel purify pSB1C3 X/S and set up ligation with that backbone and merA X/S

Methods:. After gel purifying, pSB1C3 X/S was eluted in 30 ul water.
Set up 3 ligation reactions and left at 16^0C overnight:

	5:1 reaction	3:1 reaction	- control
pSB1C3 (57.5ng/ul) (ul)	0.5	0.5	0.5
merA (11.9ng/ul) (ul)	10	6	0
10x T4 Ligase buffer (ul)	1.5	1.5	1.5
T4 DNA Ligase	0.5	0.5	0.5
Water	2.5	6.5	12.5

15 ul reactions.

Results:.
Nanodrop Data

Concentration (mg/ ul)	260/280	260/230
57.5	1.90	0.97

Need picture
Conclusion:.

/*----------*/
Page: 87

Date: 6/17/14

Title: Purification of merT for shipping vector and characterization vector, digestion of both with their respective enzymes, and repurification

Purpose: To purify merT for the shipping vector and characterization vector, to digest the shipping vector with XbaI/SpeI and the characterization vector with BglII/NotI, and to repurify it

Methods:

Purify PCR product

See pg 59 using 160 ul of buffer (B2), 40 ul of DNA, and 120V for 30min

Digest

Shipping/Characterization

XbaI/ BglII	0.5ul
SpeI/ NotI	0.5ul
NEB/ 3.1 cutter	5ul
DNA	39ul
H_2O	5ul
	50ul of each

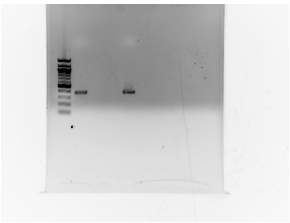
Wait for an hour

Purification

Use same kit as when purifying PCR product using 50 ul of elution buffer

Results: Characterization 3.54 ug/ml

Shipping 3.66 ug/ml



Conclusion: Ready for ligation

/*----------*/

Page: 88

Date:. 6/17/14

Title:.

Purpose:.Purify merB, run gel, digestion.

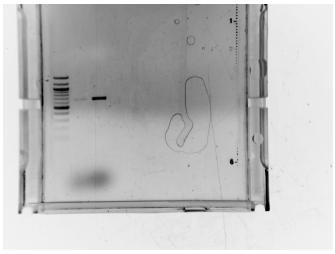
Methods:. Refer to pg. 56 and 58.
Characterization:

0.5 ul NotI
0.5 ul BglI
8.0 ul Water
6.0 ul NEB 3.1
45.0 ul DNA (merB)
60 ul total volume

Shipping

0.5 ul XbaI
0.5 ul SpeI
8.0 ul Water
6.0 ul NEB Cutsmart
45.0 ul DNA (merB)
60 ul total volume

Results:.



Conclusion:.

/*----------*/
Page: 89

Date:. 6/17/14

Title:.

Purpose:.To re-attempt ligation of merR (cut XbaI-SpeI) with shipping vector pSB1C3 for transformation into *E. coli* UQ950.

Methods:.

- Use 20 ng of shipping vector (57.5 ng/ul)
  1 ul . x 20 ~~ng~~ = 0.5 ul shipping vector
  57.5 ~~ng~~
- Setup ligation reaction (3:1)
  w. 60 ng of inert (3.03 ng/ ul)
  1 ul . x 60 ~~ng~~ = 19.8 ul insert

3.03 ~~ng~~

- Set up reaction as follows
  - 1.0 ul backbone (pSB1C3)
  - 20. ul insert (merR)
  - 2.5 ul 10x T4 DNA ligase buffer
  - 0.5 ul T4 DNA ligase
  - 2.0 ul Water
  - 26. ul rxn total
- Incubate overnight at 16^0C.

Results:.

Conclusion:.

/*----------*/
Page: 90

Date:. 6/17/2014

Title:.PCR amplification of merA from pBBRBB w/ NotI and BglII primers

Purpose:. PCr amplify merA from pBBRBB w/ bioBrick restriction sites

Methods:.
Add 249 ul NF water to merA_FBglI primer
add 326 ul NF water to merA_R NotI primer
Add 900 ul NF water to respectively labeled 1.5 ul tubes
Add 100 ul hydrated prior to labeled tubes to create 10 mmol stock
Taw 2x green mix on ice
Quantify pBBRBB: 37.8 ng/ul
Prepare 1 PCR reaction following::
- 25. ul Green mix
- 1.0 ul F. Primer
- 1.0 ul R. Primer
- 0.5 ul of HF pol
- 0.6 ul pBBRBB
- 21.9 ul NF water
- 50 ul total rxn

RXN: Partclon@ 1 hr 35 min

Results:.Purified PCR product merA BglI-NotI: 41.4 ng/ul

Conclusion:.

/*------------------------------*/
Page: 91

Date:. 6/7/14

Title:. Ligation of merT into pSB1C3

Purpose:. To ligate the PCR-amplified merT insert into the pSB1C3 shipping vector

Methods:.

Results:.

Conclusion:. The pSB1C3 shipping vector was not prepared in time to do ligation this day. See page 95

/*------------------------------*/
Page: 92

Date:. 6/17/14

Title:.

Purpose:. To purify plasmids from overnight cultures made on 6/16/14

Methods:. Follow invitrogen miniprep kit. Elute in 50 ul of Elution buffer. Label as follows
pSB1C3::ATF1 (Banana)
pSB1C3::pBBRBB GFP1
pBBRBB::GFP 2
pSB1C3::CFP 1
pSB1C3::CFP 2
pSB1C3::CFP 3

Results:.

Conclusion:.

/*------------------------------*/
Page: 93

Date:. 6/17/14

Title:.

Purpose:. To create -80^{0}C stock cultures from overnight made on 6/16/14

Methods:. Follow protocol on page 7 created pSB1C3::ATF1
pSB1C3:: CFP stocks

Results:.

Conclusion:.

/*----------*/
Page: 94

Date:. 6/17/14

Title:.

Purpose:. Transform pSB1C3-merA ligation into UQ950 and JM109

Methods:.
5 mL of ligation was used to transform JM109 with each 5:1, 3:1, and negative control for pSB1C3-merA ligation reactions. 10 ul was used for UQ950. I used the same protocol on page 29. I added LB to JM109 cells and RB to UQ950 cells. I plated on Cm plates (100 ul) only after centrifuging the samples @ 7,000 g for 1 min. I left the plates in a 37^{0}C incubator overnight in Claudia Schmidt-Dannert lab.

Results:. No growth on any plates.

Conclusion:.

/*----------*/
Page: 95

Date: 6/18/14

Title: : Ligation of merT and shipping vector

Purpose: To ligate pSB1C3, the shipping vector, and merT together

Methods:
ug DNA x $\frac{\text{pmol}}{10^6 \text{ pg}}$ x $\frac{1}{\quad}$ = pmol

660 pg 1 ug # nucleotides

Shipping vector = .04810 ug ---> 0.035 pmol (2070 bp)
Mer T = .00366 ug ---> 0.016 pmol (351 bp)

Mix the following together and let incubate @ room temp for 1 hr.

Shipping vector	2ul
Mer T	14ul
T4 Ligase	1ul
Ligase Buffer	2ul
H_2O	1ul

Results: MerT was ligated into the shipping vector.

Conclusion: pSB1C3::merT is ready to transform into *E. coli*.
/*----------*/
Page: 96

Date:. 6/18/14

Title:.

Purpose:. Amplify merA and set up overnight digest w/ XpaI and SpeI

Methods:.

- Used volumes on page 51 for PCR. merA was amplified from pBBR-BB merRTPAB
- Ran on a DNA gel and gel purified it; eluted in 34 ul of water
- Set up overnight restriction digest:
  1 ul SpeI
  1 ul XbaI
  4 ul CutSmart buffer
  34 ul PCR product
  40 ul Total

Left in 37^0C incubator overnight

Results:. Need picture

Conclusion:.

/*----------*/
Page: 97

Date:. 6/18/14

Title:.

Purpose:.To transform *E. coli* UQ950 with pSB1C3_merR ligation mixture.

Methods:.
See page 29*
*Modification
- Use LB + cm plates
- Only one ratio used (3:1) so use only 2 plates

Incubate at 37^0C overnight
Run ligation mixture on gel to determine success of ligation

Results:. Need picture
No growth on either plates. :-(

Conclusion:.

/*----------*/
Page: 98

Date:. 6/18/14

Title:.

Purpose:. merB/pSB1C3 ligation (X/S digested)

Methods:.
1. Measurer merB X/S conc. --- 0.0908 ng/ul
2. Make the reaction mix
   Shipping vector pSB1C3 @ 48.1 ng/ul --- 1 ul
   merB PCR product (too low)

Results:.

Conclusion:. merB (X/S) conc. too low; reprep merB PCR (X/S)

/*----------*/
Page: 99

Date:. 6/18/14

Title:.

Purpose:. merB PCR

Methods:.

1. Reaction mix
   1 ul pDU1358
   0.5 ul merB RBB @ 10mM
   0.5 ul merB FBB @ 10mM
   25 ul Go Taq (2x)
   22.5 ul NF water
   1 ul HF DNA polymerase

2. Run PCR using protocol on p51

Results:.

Conclusion:.

/*----------*/
Page: 100

Date: 6/19/14

Title: Transformation of *E. coli* strain UQ950 with pSB1C3::merT

Purpose: To transform *E. coli* with our ligation product

Methods: See page 29

Results: No colonies formed.

Conclusion: Reattempt amplification in order to ligate that into a new shipping vector.

/*----------*/
Page: 101

Date:. 6/19/14

Title:.

Purpose:. To amplify merR isomg {CR

Methods:. See page 61

Modifications
10mM stocks already prepared
See page 63 for gel protocol (120V for 35 min)

Results:. Need Picture
DNA concentration 24.1 ng/ul
Conclusion:.

/*----------*/
Page: 102

Date:. 6/19/14

Title:.

Purpose:. Purify merA digest, ligae with pSB1C3 X/S and transform into JM109.

Methods:. Purified merA X/S and eluted into 30 ul

20 ul ligation reaction
1 ul pSB1C3 X/S (48.1 ng/ul)
11 ul merA X/S (11.7ng/ul)
1 ul T4 Ligase
2 ul T4 Ligase buffer
5 ul water

Left at room temp for 1 hr.
Transformed 10 ul into JM109 and 10 ul into UQ950 following the protocol on page 94. Left both cm plates in the 37^0C incubator overnight in the CSD lab.

Results:.
merA concentration 11.7 ng/ul

Conclusion:.

/*----------*/
Page: 103

Date:. 6/19/14

Title:.

Purpose:. merB (X/S) + pSB1C3 (X/S) ligation

Methods:.
Use molar ratio of pSB1C3-merB (1:3)

pSB1C3 @ 48.1 ng/ul (2070 bp) = 0.035 pmol/ul
merB (X/S) @ 7.78 ng/ul (639 bp) = 0.018 pmol/ul

Scale up to pSB1C3:merB = 0.070 pmol: 0.210 pmol (1:3)

1. Make rxn mix
   2 ul pSB1C3
   11.67 ul merB
   1 ul T4 Ligase
   2 ul T4 Ligase buffer
   3.33 ul NF water
   20 ul Total

2. RT incubation for 1 hr

Results:.

Conclusion:.

/*----------*/
Page: 103

Date:. 6/20/14

Title:.

Purpose:. Restriction digest to confirm plasmid existence

Methods:.
1. Make rxn mix
   10 ul ligation product (~7.00 bp) max 0.035 pmol ~0.06ug or 60 ng
   2 ul of NEB buffer
   0..5 ul PstI
   7.5 ul water
2. Incubate @ 37^0C for 1 hr
3. Run digest on 1% agarose gel (ladder: 1 kb)

Results:.Need picture

pSB1C3 backbone@ 2 kb
No bands around 0.5 kb or 2.7 kb
primer dimerization <0.5 kb

Conclusion:. merB didn't amplify back in initial PCR need to redo rxn.

/*----------*/
Page: 104

Date:. 6/20/14

Title:. Ligation of merP to shipping vector

Purpose:. To Ligate merP to pSB1C3

Methods:.
merP @ 5.2 ng/ul = 0.058 pmol/ul
pSB1C3 = 0.035 pmol/ul

3:1

2 ul pSB1C3
7.24 ul merP
1 ul T4 Ligase
2 ul T4 Ligase buffer
7.76 ul water
20 ul Total

5:1

2 ul pSB1C3
12 ul merP
1 ul T4 Ligase
2 ul T4 Ligase buffer
3 ul Water
20 ul Total

Incubate at room temp for 1 hour

Results:.

Conclusion:.

/*--------------------------------*/

Page: 105

Date: 6/23/14

Title: Ligation of merT into the characterization vector pBBRBB

Purpose: To ligate merT into pBBRBB

Methods:

ug DNA x $\frac{\text{pmol}}{660\text{ pg}}$ x $\frac{10^6\text{ pg}}{1\text{ ug}}$ x $\frac{1}{\#\text{ nucleotides}}$ = pmol

Characterization vector = .02400 ug ---> 0.0176 pmol (4100 bp)

Mer T = .00354 ug ---> 0.0153 pmol (351 bp)

Mix the following together and let incubate @ room temp for 1 hr.

Characterization vector	2ul
Mer T	7ul
T4 Ligase	1ul
Ligase Buffer	2ul
H_2O	1ul

Results: Ligation complete.

Conclusion: Ready to transform *E. coli*. UQ950 with pBBRBB::merT ligation product

/*--------------------------------*/

Page: 105

Date: 6/23/14

Title: Mini-prep of pBBRBB

Purpose: To purify plasmid pBBRBB

Methods: Followed instructions from the kit except

5. Bind 2 columns instead of 4
7. Wash twice w/ 0.5 vol of W9 and final spin 12,000 g for 2 min
8. Elute w/ H_2O and incubate for 10 min

Results: pBBRBB was purified.

Conclusion: pBBRBB is ready for use.

/*----------*/
Page: 112

Date: 6/23/14

Title: PCR colony screen of transformants with merT

Purpose: To isolate some colonies that have merT

Methods:
Select 4 isolated colonies, create patch plate w/ each colony, and add the following to each colony in addition to positive control (0.5 ul of pDU1358)

5ul GoTaq
4ul H_2O
0.5ul F. primer BglI (10nM)
0.5ul R. primer NotI (10nM)

Perform PCR. Annealing temperature of 54^0C was used with protocol on pg 15

Results: Patch plate yielded no growth

Conclusion: Due to lawn on plate, retransform more *E. coli* to replate.

/*----------*/
Page: 106

Date:. 6/23/14

Title:.

Purpose:. To digest merR with BglI and NotI for ligation into the characterization vector pBBRBB

Methods:.
Follow protocol optimizing restriction endonucleases reactions from NEB (in iGEM 2014 protocol binder).

merR digest
0.5 ul BglI
0.5 ul NotI
40 ul DNA (merR)
5 ul 10x NEBuffer
4 ul dd water

50 ul Total

Combine and incubate @ 37^0C for 1 hr.
Purify with invitrogen kit and store @ -20^0C

Results:.

Conclusion:.

/*----------*/
Page: 107

Date:. 6/23/14

Title:.

Purpose:. Set up gel to check the right orientation of psB1C3:merB

Methods:.
Run Steve' sample merB-pSB1C3, PCR w/ sequencing primer: labelled #1-10 on 1% agarose gel for 30 min (20V)

Results:.

Conclusion:.

/*----------*/
Page: 108

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 109

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 110

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 111

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

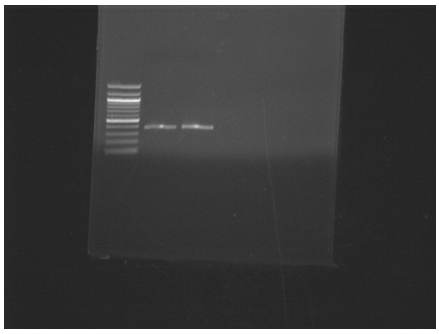
/*----------*/
Page: 112

Date: 6/24/14

Title: Re-amplify merT for shipping vector and perform gel electrophoresis

Purpose: Reamplify merT in order to reattempt ligation and to perform a gel electrophoresis to verify PCR worked

Methods: See pg 59 letting the gel run for 40 min @ 120V

Results: 

Conclusion:

/*----------*/
Page: 113

Date:. 6/24/14

Title:.

Purpose:.Transform pBBRBB-merA into JM109 and plate on km plates

Methods:.See page 29 for transformation protocol methods. Used 50 ul JM109 cells for pBBRBB-merA 5:1 ad 3:1 for JG and CSD ligations. Spin down at 7,000 g for 1 min. Resuspended in 50 ul LB + plate on km plates. Left in CSD lab 37^0C shaker.

Results:.

Conclusion:.

/*----------*/
Page: 114

Date:. 6/24/14

Title:.

Purpose:. To perform two ligations with merR (cut BglI and NotI) and pBBRBB (cut BglI and NotI) (characterization vector)using buffers/ enzymes from the Gralnick lab and CSD lab separately for transformations into *E. coli*.

Methods:. 3:1
1 ul backbone
5 ul insert
1 ul T4 ligase buffer (10x)
0.5 ul T4 Ligase
2.5 ul water
10 ul total

Use same recipe using enzymes/ buffers from each lab separately (Gralnick lab ligations set u first, hen CSD lab ligation)

Incubate both overnight @ 16^0C from transformations next time.

Results:.

Conclusion:.

/*----------*/
Page: 115

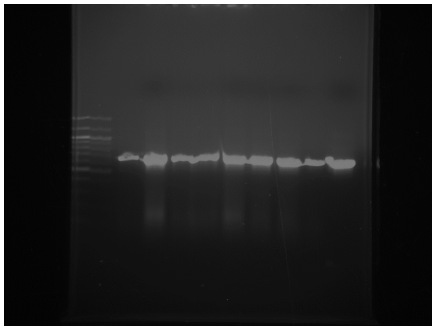
Date:. 6/24/14

Title:.

Purpose:. Purify DNA from the gel ran by Sarah on page 112 and Run a gel w/ merB, control form colonies.

Methods:.
Only able to recover pSB1C3

Results:. Concentration 32.9 ng/ul



Conclusion:.

/*----------*/
Page: 116
Date:. 6/24/14

Title:.Preparation of 1% Agarose + EtBr for Gel Electrophoresis

Purpose:. To prepare more 1% Agarose + Ethidium Bromide mixture for pouring a gel for gel electrophoresis tests.

Methods:.
When no more liquid mixture remains in the 1% agarose + EtBr bottle, remove the bottle from the 60^0C incubator or refilling, and follow the protocol below for refilling:

- At the weighing station, measure out 4 g of solid agarose (1% of a 400 ml mixture) Using a scale, filler paper folded into quadrants, and the scoop attached of the agar bottle (or a clean scoop if none is attached).
- Carefully pour the solid agar into the empty 1% agarose. EtBr bottle
- next to the gel electrophoresis station (Gralnick lab), measure out 400 ml of TAE buffer into a graduated cylinder and pour it into the bottle
- Swirl gently to submerge all of the agar
- Microwave the bottle with the cap removed fro 5-6 minutes or until the agar is completely dissolved in the mixture. Remove the bottle every minute or so to swirl in the mixture, being careful to wear a glove or the red lobster grip to avoid burns, so that the agar dissolved and so that the liquid does not boil over, swirl to mix
- Replace the cap on the refilled bottle
- When the agar is dissolved, add one (1) drop of ethidium bromide to the bottle, and replace the bottle in the 60^0C for future use.

Results:. 1% agarose-EtBr mixture sis read for future use.

Conclusion:.

/*------------------------------*/
Page: 117

Date:. 6/24/14

Title:. Ligation of merA into pSB1C3

Purpose:.Ligate mer A insert into pSB1C3 shipping vector using previously digested pSB1C3 and merA

Methods:.
8.5 ul merA X/S (11.7 ng/ul)
1 ul pSB1C3 (32.9 ng/ul)
1 ul T4 Ligase
2 ul T4 Ligase buffer
7.5 ul water
20 ul total

Keep overnight in 16^0C incubator

Results:.

Conclusion:.

/*------------------------------*/
Page: 118

Date:. 6/24/14

Title:. PCR amplification of merT

Purpose:. To PCR amplify merT for later insertion into the pSB1C3 shipping vector

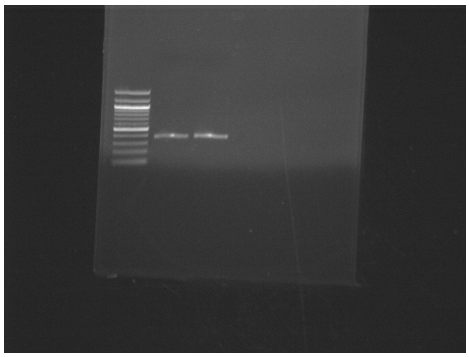
Methods:.
Two PCR tubes samples of merT DNA were obtained from the iGEM 2014 freezer box (prepped by Sarah earlier in the day).

PCR was performed according to the following protocol:

1	2	3	4	5	6	7

95^0C	95^0C	54^0C	72^0C	steps 2-4	72^0C	10^0C
2:00	0:30	0:30	1:30	28x	5:00	oo
Denature		Anneal	Extend		Final Extension	

Results:. PCR samples were stored in the freezer in the Gralnick lab for gel test, purification, and future use for insertion into the shipping vector



Conclusion:.

/*----------*/

Page: 119

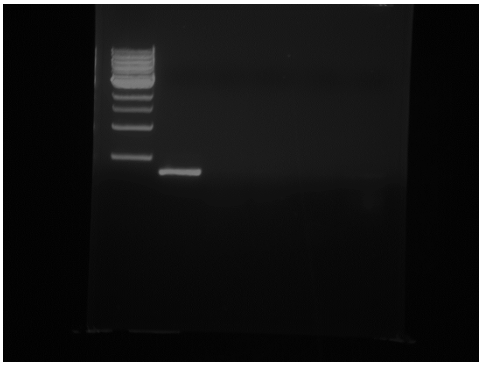
Date:. 6/24/14

Title:.Gel Electrophoresis test of pBBR-BB:: merT transformants

Purpose:. To test potential transformants of *E. coli* containing pBBR-BB::merT picked from a transformant plate (see p. 111 of lab notebook) on a gel by SDS-PAGE.

Methods:.

The protocols on page 59 and 116 were used. For the page 59 protocol, only 8 ul of the 1 kb ladder were used instead of 10 ul. The gel was run at 120V for ~40 min.

Results:. 

Conclusion:. The colonies tested did not contain the merT insert

/*----------*/

Page: 120

Date: 6/25/14

Title:. Reattempt ligation for PCR colony screen

Purpose:. Reattempt ligation in order to perform the PCR colony screen again

Methods:.

Ligation into PCR tube

2ul pBBRBB *see pg 105 for calculations
7ul merT*
1ul T4 Ligase buffer
2ul Ligase
8ul H_2O
10ul mixture

Incubate @ room temperature for an hour.

Prepare master mix for PCR colony screen

5 ul Go Taq
4 ul H_2O
0.5ul F. Primer BglI
0.5ul R. Primer NotI
10ul Mixture x 7 for 5 colonies, 1 +control, and 1 extra

Results:.Ligation and Master Mix prepared

Conclusion:.PCR tube labelled merT and ligation on side located in 16^0C freezer. Ready for transformation. M. mix located in unmarked iGEM box.

/*------------------------------*/
Page: 120

Date:. 6/25/14

Title:. Gel of MerT for shipping vector

Purpose:. To run a gel of merT using the shipping vector primers and to quantify it

Methods:.See pg 59 @120V for 35 minutes
Combined 2 45ul into 1 purification
used 50ul H_2O instead of elution buffer

Results:. Need picture

Conclusion:.17.7 ug/ml of a 49 ul sample quantified using Qubit Assay. Sample ready for digest and located in unmarked iGEM box.

/*------------------------------*/
Page: 121

Date:. 6/25/14

Title:.

Purpose:. To transform *E. coli* using two different methods with pBBRBB_merR ligation mixtures

Methods:.
Set up four total transformations
Using 10 u from the CSD lab ligation and 10 ul from the Gralnick lab ligations separately follow heat-shock transformations method on page 29, using four plates
CSD high and low and Gralnick high and low

Results:.

Conclusion:.

/*------------------------------*/
Page: 122

Date:. 6/25/14

Title:. Purification and digestion of merP BglI and NotI

Purpose:.

Methods:.
Purification kit (25 ul)

Digestion
25 ul PCR product
3 ul CutSmartf buffer
0.5 ul BglI
0.5 ul NotI
1 ul water
30 ul total

Incubate at 37^0C for 1 hour

Purification 25 ul elution

Results:.

Conclusion:.

/*----------*/
Page: 123

Date:. 6/25/14

Title:.

Purpose:. Start 2 4 ml cultures of pBBRBB-merA for miniprepping and sequencing tomorrow. Also banana-pSB1C3 vector and set up a digest. Transformed pSB1C3 mini-prepped into JM109.

Methods:.
Took 2 different colonies of 5:1 CSD (6/24) plate.
Rxt the plates in the Gralnick lab fridge.

Set up 2 restriction digest reactions w/ psB1C3_banana:

50 ul vector
1 ul SpeI

1 ul XbaI
11 ul water
7 ul CutSmart
70 ul total

Also started colony pcr with 3 colonies from each: pBBRBB-merA CSD 5:1, 3:1
JG 5:1, 3:1
5 ul Water
5 ul GG
0.3 ul each primer
tip of colony

Left in PCR cycler overnight in CSD lab.

See page 29 for transformation protocol. Left in 37^0C CSD incubator only plate after centrifugation.

Results:.

Conclusion:.

/*----------*/
Page: 124

Date:. 6/26/14

Title:. Digestion and Transformation of MerT for shipping vector

Purpose:. To digest merT for shipping vector and transform it into *E. coli* strain UQ950

Methods:.
Method 1
XbaI 0.5ul
SpeI 0.5ul
NEB 5 ul
merT 49 ul
H_2O 5 ul
60ul
Let incubate for 1 hour @ 37^0C incubator
Purify w/ same kit as PCR clean-up kit
except with 10 min incubation and 25 ul of H_2O

Method 2
See page 29

Results:.
Digest completed. On the more plate there were 104 colonies, and the less plate had 12 colonies.

Conclusion:.merT is ready to be ligate w/ the shipping vector. It is located in unmarked iGEM box in -20^{0}C cooler. Master mix is already prepared. Colonies are ready for colony PCR screen.

/*----------*/
Page: 125

guDate:. 6/26/14

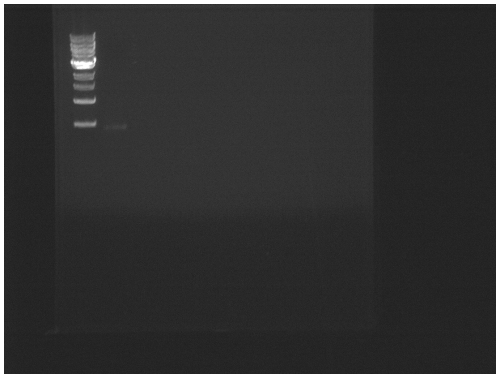
Title:.

Purpose:. PCR amplify merR for transformation into characterization vector pBBRBB

Methods:.
Use protocol from page 51

1 ul pDU1358
0.5 ul merR_F_BglI
0.5 ul merR_R_NotI
25 ul GoTaq mix
22.5 ul water
0.5 ul DNA polymerase
50 ul total

Run on a gel to confirm amplification (5 ul). Clean up PCR product w/ invitrogen kit.

Results:. 

Conclusion:.

/*----------*/
Page: 126

Date:. 6/26/14

Title:.

Purpose:. .Colony PCR on eight colonies from pSB1C3-merA plate from page 123, then ran DNA on a gel.

- Ran a colony PCR from page 123 (pBBRBB-merA) on a gel
- Dephosphorylation of pSB1C3-banana digest from page 123. Then gel purified
- Miniprepped the two colonies from page 123, since both were confirmed w/ colonies

Methods:.Colony PCR

5 ul Water
5 ul GG
0.3 ul each primer
tip of colony

See page 296 for dephosphorylation protocol:

8 ul antarctic phosphate buffer
1 ul antarctic phosphatase
1 ul water

for both digested reactions (70 ul each)
Incubated for 30 min @ 37^0C
Left at 70^0C for 5 min, room temp 5 min
Ran on gel and purified

Made a -80^0C stock of pBBRBB-merA (JM109), see page 7 for protocol

Results:. need picture

Conclusion:.

/*----------*/
Page: 127

Date:. 6/27/14

Title:.

Purpose:. pSB1C3-mer ligation & transformation (*E. coli* UQ950)

Methods:.
Make rxn mix

1 ul pSB1C3 (X/S) @ 48.1 ng/ul
18.5 ul merT (X/S) @ 8.47 ng/ul
1.0 ul T4 Ligase
2.3 ul T4 Ligase buffer
22.8 ul Total

*Molar ratio of backbone = insert = 1:2

RT(room temp) incubation for 1 hr
Take 10 ul and perform transformation listed on page 29. Put rest into 16^0C overnight incubation

*protocol modification
-ice incubation for 50 min because water bath was off
-prepare two plates; 75 ul → 150 ul media modification to prepare enough aliquot

Results:.

Conclusion:.

/*----------*/
Page: 128

Date:. 6/27/14

Title:.

Purpose:. pBBRBB-mer X/S digestion

Methods:.

1. Mix

   35 ul pBBRBB- merO @24.2 ng/ul
   10 ul NEBuffer (cutsmart)
   2 ul XbaI
   2 ul SpeI
   51 ul water
   100 ul Total

2. Overnight incubation @ 37^0C

Results:.

Conclusion:.

/*----------*/
Page: 129

Date:. 6/27/14

Title:.Ligations of merP to pBBRBB

Purpose:.

Methods:.
Vector = 24 ng/ul, 4738 bp = 0.004 pmol/ul
insert = 27.5 ng/ul, 270 bp = 0.75 pmol

3:1
2 ul Vector
11 ul insert
0.5 ul Ligase
2 ul buffer
4.5 water
20 ul

5:1
2 ul Vector
6.5 ul insert
0.5 ul Ligase
2 ul buffer
20 ul

Results:.

Conclusion:.

/*----------*/
Page: 130

Date:.6/27/14

Title:.

Purpose:. Miniprep pSB1C3-merA (1) + (4)

Methods:.
Use invitrogen kit t miniprep

Results:.
Concentrations pSB1C3-merA (1): 64.0 ng/ul
(4): 43.7 ng/ul

Concentrations pBBRBB-merA (1): 21.5 ng/ul
(2): 20.4 ng/ul
Conclusion:. Left all samples in iGEM box in cold room

/*----------*/
Page: 131

Date:. 6/30/14

Title:. PCR colony screen

Purpose:. PCR colony screen select transformants w/ merT

Methods:.Use Master mix (see pg 120) and create more for added colonies.

Prepare master mix for PCR colony screen
5 ul Go Taq
4 ul H_2O
0.5ul F. Primer BglI
0.5ul R. Primer NotI
10ul Mixture x 3 for 3 addition colonies

Perform PCR. Anneal @ 54^0C (see pg 15)
Prepare patch plate screen to verify *E. coli*. Let incubate overnight.

Results:. Ready for gel verification

Conclusion:. Wait for patch plate to grow and perform gel electrophoresis to verify merT

/*----------*/
Page: 131

Date:. 6/30/14

Title:. Ligation of shipping vector and merT

Purpose:. To ligate pSC1C3 with merT

Methods:.

$$\text{ug DNA} \times \frac{\text{pmol}}{660\ \text{pg}} \times \frac{10^6\ \text{pg}}{1\ \text{ug}} \times \frac{1}{\#\ \text{nucleotides}} = \text{pmol}$$

Shipping vector = .0329 ug ---> 0.0241 pmol (2070 bp)
Mer T = .0309 ug ---> 0.133 pmol (351 bp)

Mix the following together and let incubate @ room temp for 1 hr.

Shipping vector	2ul
Mer T	1.1ul
T4 Ligase	1ul
Ligase Buffer	2ul
H_2O	13.9ul
	20 ul for 2 transformations

Incubate @ room temperature for 1 hr in PCR tube
Results:. Ligation complete

Conclusion:.PCR tube labelled merT X/S and ligate on side located in 16^0C freezer. Ready for transformation. M. mix located in unmarked iGEM box.

/*----------*/
Page: 132

Date:. 6/30/14

Title:.

Purpose:. Inoculate pBBRBB::mer for mer digest
Inoculate pBBRBB::merA for merAB digest
Inoculate pBBRBB::merB for merAB digest

Methods:.

1. Set up tglass tubes w/ 5 uml LB+ 5 ul kan each
2. Pick colonies:
   - 6 colonies from pBBRBB:mer
   - 3 colonies from pBBRBB:merA(LSD). #1-3
   - 3 colonies from pBBRBB:merB #2-4
3. Overnight incubation in 37^0C

Results:.

Conclusion:.

/*----------*/
Page: 133

Date:. 6/30/14

Title:. Transformation of potential pSB1C3::merT into competent UQ950

Purpose:.To plate the ligation of pSB1C3 with merT after digestion from X-->S in order to determine if the ligation was successful by transforming UQ950 *E. coli* cells and plating them.

Methods:.
Transformation of UQ950 cells with pSB1C3::merT was done following the procedure on page 111 with the following modifications to the plating: a more concentrated and less concentrated plates were plated. The procedure on page 29 was followed. Transformed cells were plated on LB + cm plates.

Results:.
A more concentrated plate and the less concentrated plate were incubated in the 37^0C incubator overnight viewing on 7/2.

More: lawn
Less: ~89

Conclusion:.

/*----------*/
Page: 134

Date:. 6/30/14

Title:. Gel Electrophoresis of potential pBBRBB::merT cells

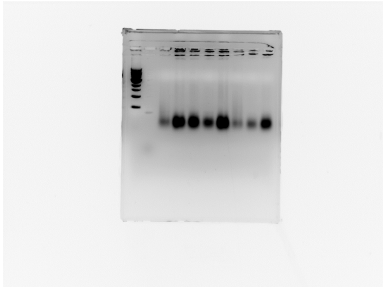
Purpose:. To test colonies from the PC colony screen of pBBRBB::merT transformants

Methods:.
Gel Electrophoresis apparatus and samples were prepared/ loaded according to the steps on page 59.

The samples were loaded into ten wells in the following order and run at 120V for 30 minutes

Wells	1	2	3	4	5	6	7	8	9	10
Sample	1 kb ladder	+ control	more 1	more 2	more 3	more 4	less 1	less 2	less 3	less 4

Results:.



Conclusion:. No definitive bands were seen. the PCR screen should be repeated if colonies/ plates were saved

/*----------*/
Page: 135

Date:. 6/30/14

Title:. Amplifying merP for insertion into the shipping vector

Purpose:. Can then submit merP to iGEM

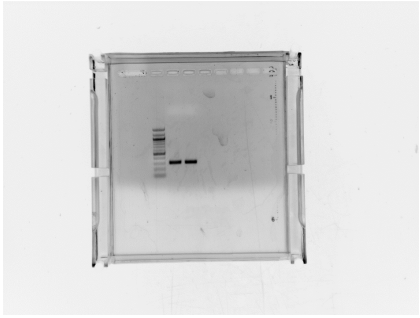
Methods:.
PCR in thermocycler

1 ul pDU1358
0.5 ul merP F. primer
0.5 ul merP R.primer
25 ul Go Taq polymerase
22.5 ul water
0.5 ul NF pFU ultra poly.
50 ul Total x 2 samples

	1	2	3	4

Temp (^{0}C	95	95	54	72
Time (min)	2:00	0:30	0:30	1:00

Repeat 29x



Results:.
After clean-up
Concentrations
13.0 ng/ul
14.0 ng/ul

Conclusion:.

/*----------*/
Page: 136

Date:. 6/30/14

Title:. Logged in pBBRBB-merA (1) into gram database

Purpose:.

- Start 2 4 ml culture of pSB1C3-merA (1) to make a 1 ml -80^{0}C stock the next day.
- PCR check to see if pSB!c# merA colonies are in correct orientation

Methods:.
For PCR check:
5 ul GG
5 ul water
0.3 ul pSB1C3 F.primer
0.3 ul merA R. primer
3 ul miniprepped DNA from pSB1C3 (1)/ (4) or colony from plate

Results:. Need picture

Conclusion:.

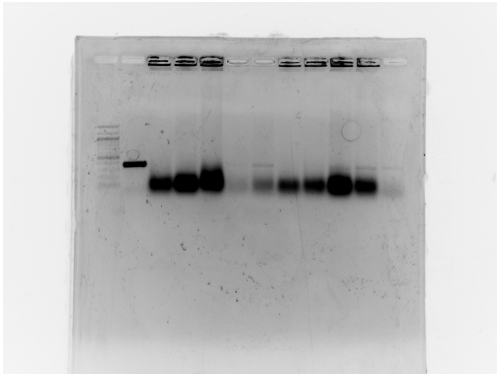
/*----------*/
Page: 137

Date:. 7/1/14

Title:. Reattempt PCR colony purification of MerT

Purpose:. Redo PCR colony purification to get better gel results

Methods:. Prepare Master Mix
5 ul Go Taq
4 ul H_2O
0.5ul F. Primer BglI
0.5ul R. Primer NotI
10ul Mixture x 12 for 5 more colonies, 5 less colonies, 1 +control and 1 extra
Perform PCR
Prepare patch plate
Perform gel for verification

Results:. 

Conclusion:. Colonies less 5, more 3, more 4, and more 5 have been identified as having merT. Make overnight cultures using regrown colonies from patch plate.

/*----------*/
Page: 138

Date:. 7/1/14

Title:.

Purpose:. To digest merR PCR product BglI/NotI for ligation into characterization vector pBBRBB

Methods:. Follow protocol on page 106

Results:.

Conclusion:.

/*----------*/
Page: 139

Date:. 7/1/14

Title:.

Purpose:. Digest X/S merP

Methods:.
74 ul merP @ 13.5 ng/ ul
0.5 ul XbaI
0.5 ul SpeI
5 ul 10x NEBuffer
20 ul dd water
100 ul Total

Incubate at 37^0C for 3 hrs

Results:.

Conclusion:.

/*----------*/
Page: 140

Date:. 7/1/14

Title:.

Purpose:. To miniprep and BglII digest of pBBRBB-mer/merA/merB

Methods:.

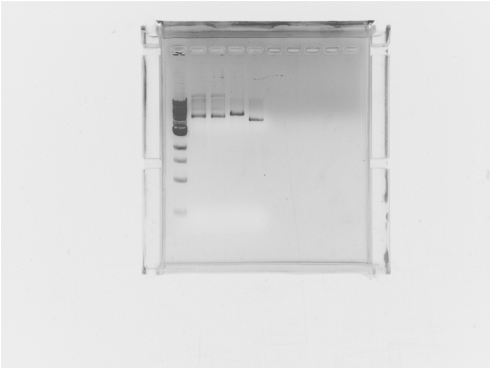
1. Perform miniprep sin Invitrogen Quick miniprep kit

products- mer1 \
mer-2 \ pBBRBB::
merA /
merB /

2. Measure DNA quantify
3. Perform BglL digest
   10 ul vector
   1.5 ul 3.1 buffer
   0.5 ul BglI
   3.0 ul Water
4. Incubate @ 37^0C 1 hr
5. Ru on 1% gel and observe

*Expected Bands
pBBRBB-mer: 4.1 kb + 3.5 kb = 6.9 kb
pBBRBB-merA: 4.1 kb + 0.6 kb = 4.7 kb
pBBRBB-merB: 4.1 kb + 1.7 b = 5.8 kb

Results:. Not consistent w/ expectations ladder might be contaminated → run a double digest to confirm bands
mer operon → correct
merA→ looking weird
merB---> correct



Conclusion:.

/*----------*/
Page: 141

Date:. 7/1/14

Title:.

Purpose:.
Set up overnight restriction enzymes digest of pBBRBB-merA (EcoRI and SpeI) and colony pBBRBB-merB (EcoRI and XbaI)
Logged in pSB1C3-merA #1 into database

Methods:.
Restriction Digest:
34 u plasmid
1 ul each restriction enzyme
4 ul CutSmart

Left in 37^0C incubator overnight.

Results:.

Conclusion:.

/*----------*/
Page: 142

Date:. 7/2/14

Title:. PCR colony purification w/ pSB1C3::merT and prepare overnight cultures of pBBRBB::merT

Purpose:. PCR colony purification w/ pSB1C3::merT and to create overnight cultures

Methods:.
Method 1
Use mix prepared (see pg 131).
Perform PCR.
Prepare patch plate

Method 2
See pg 5-6

LB 5 ml
kan 5 ul
colony swab

Results:. PCR product ready for gel verification and overnight cultures ready.

Conclusion:. PCR product located in freezer in Gralnick lab. Cultures are stored in 37^0C shaker and will be ready for miniprep.

/*----------*/
Page: 143

Date:. 7/2/14

Title:.

Purpose:. To digest pBBRBB BglI/NotI for ligation with other parts for characterization, to ligate merR cut X/S with pSB1C3 cut X/S for transformation into *E. coli*, and to ligate merR cut B/N with pBBRBB cut B/N for transformation into *E. coli.*

Methods:.
1. Follow protocol on page 26 using BglI and NotI instead of XbaI and SpeI

2. Set up single ligation according to protocol on page 114

3. Follow protocol on page 114 with following recipe

2 ul backbone
6 ul insert
1 ul 10x T4 Ligase buffer
0.5 ul T4 Ligase
0.5 water
10 ul total volume

Incubate overnight @ 16^0C for transformation next time.

Results:. Need picture

Conclusion:.

/*----------*/
Page: 144
Date:. 72/14

Title:. transformation of pBBRBB::erP and pSB1C3 into UQ950

Purpose:.

Methods:. The procedure was performed following instructions on page 29.

For the shipping vector LB+ cm plates were used
For both plasmids 1:10, 1:100, and 1:1000 were plated.

Results:.

Conclusion:.

/*----------*/
Page: 145

Date:. 7/2/14

Title:.

Purpose:.Run the pBBRBB-merA (EcoRI/ SpeI) and BBRBB-merB (EcoRI and XbaI) Restriction digest on a DNA gel
Setup ligation and plated on Kb plates

Methods:. Gel purified the pBBRBB-merB backbone and merA insert, concentrations were very low (8.6 ng/ul and 5.3 ng/ul, respectively), set up a 1 hr @ room temp ligations:

14 ul merA
7 ul pBBRBB-merB backbone
0.5 ul water
2.5 ul T4 ligase buffer
1 ul T4 ligase
25 ul reactions

Transformed JM109 with 15 ul reaction and left in incubator (37^0C) overnight (See protocol on page 29.)

Results:.Need picture

Conclusion:.

/*----------*/
Page: 146

Date:. 7/3/14

Title:. Miniprep of *E. coli* and Gel verification for merT

Purpose:. To miniprep *E. coli* and use a gel to verify merT was in pcr product

Methods:.
Method 1
Followed directions from kit except
50ul of H_2O instead of 75ul elution buffer
incubated for 10 min instead of 1 min
Method 2
See pg 120 using 120V for 35 min

Results:. Used Qubit Assay to quantify
m5 2.12 ug/ml
m4 2.46 ug/ml
m3 too low reading
l5 too low reading

need picture

Conclusion:.Restart both liquid cultures on Monday.

Date:.

Title:.

Purpose:.

Methods:.

Results:.

Conclusion:.

/*----------*/
Page: 147

Date:. 7/3/14

Title:. Screen PCR for merB transformation

Purpose:.

Methods:.
PCR
10 ul GoTaq

0.5 ul merP F. BglI
0.5 ul merP R. NotI
9 ul water
<u>- cells</u>
20 ul

Results:. Need picture
1-10 single colonies in a 1:1000 plate LB+ Kan
11 single cell colonies in a LB+ Kan
13 group of cells in LB + Kan
14, 15 group of cells in LB + Cm

Conclusion:.

/*----------*/
Page: 148

Date:. 7/3/14

Title:.

Purpose:.To transform *E. coli* w/ pBBRBB merR and with pSB1C3_merR (separately).

Methods:. Follow protocol on page 29 using kan plates for pBBRBB-merR and Cm for pSB1C3_merR

pBBRBB	kan	high
pBBRBB	kan	low
pBBRBB	(LB)+ control	low
pSB1C3	cm	high
pSB1C3	cm	low
pSB1C3	(LB) + control	low

*Used 2 tubes of UQ950 cells

Results:.
Growth on all plates. Controls were fields, lots of growth on cm plates, and few dozen colonies on kan plates ---> move on to colony screen

Conclusion:.

/*----------*/
Page: 149

Date:. 7/3/14

Title:.

Purpose:. PCR colony screen the pBBRBB-merAB (JM109) transformations from page 145

Methods:. 5 colonies were present on the plate. PCR react as follows
5 ul water
5 ul GG
0.3 ul merA F. Primer
0.3 ul merA R. Primr
tip of colony

Left reaction in cycler (CSD) overnight.

Results:.

Conclusion:.

/*----------*/
Page: 149

Date:. 7/4/14

Title:.

Purpose:. Run colony PCR on a DNA gel from above

Methods:.Ran for 25 min, 100V

Results:. Results for 5 colonies of pBBRBB-merAB(JM109):
need picture
(only colony four didn't work.)

Conclusion:.

/*----------*/

Page:. 150

Date:. 7/714

Title:. Create overnight cultures for characterization vector and merT and shipping vector and merT

Purpose:. To create overnight cultures of *E. coli* containing our respective ligation product

Methods:.
Method 1
See pg 5-6

LB 5ml
cm/kan 5ul
colony swab
Method 2
Create M. Mix for PCR colony screen of more colonies
Prepare master mix for PCR colony screen

5 ul Go Taq
4 ul H_2O
0.5ul F. Primer
0.5ul R. Primer
10ul Mixture x 8 characterization and x10 for shipping vector

Results:. M. Mix prepped. Overnight cultures ready.

Conclusion:. Cultures are in 37^0C shaker, and ready to be mini-prepped tomorrow. PCR tubes containing m. mix are located in freezer in lab space.

/*----------*/
Page:. 151

Date:. 7/7/14

Title:.

Purpose:. PCR colony screen of pBBRBB-merR and pSB1C3-merR transformations to verify successful transformation.

Methods:.
Prepare master mix:

5 ul Go Taq
4 ul Water
0.5 ul F. primer (BglI)
0.5 ul R. primer (NotI)
10ul total x 15 for 12 colonies and 1 +control

Aliquot 10 ul into PCR tubes and spot colony into tube w/ small pipette tip. Incubate ~ 10 min and swirl tip in tube. Spot tip onto labeled spot plate (incubate 37^0C overnight).

Run PCR on samples with colony program.
Run samples on gel to verify positives.

Results:.
Need picture
Gel Order

pBBRBB	1 kb	1	2	3	4	5	6
		+	-	-	+	-	+
pSB1C3	1 kb	1	2	3	4	5	6
		-	-	-	-	-	-

Negative control worked.

Conclusion:.

/*----------*/
Page:. 152

Date:. 152

Title:. Verify sequence of pSB1C3-merB

Purpose:.

Methods:. Prepare 6.4 uM primer stock.
Prepare sequencing reactions for colony 2, 3, 5. x3 rxns

Colony	Concentrations (ng/ul)
2	166
3	172
5	216

Each sample has 1 ul of 6.4 picoM, 400 ng vector, and NF water up to 12 ul

Colony	Sch	Primer
2	7	pSB1C3 seq F
2	8	merB seq 1F
2	9	pSB1C3 seq R
3	10	pSB1C3 seq F
3	11	merB seq 1F
3	12	pSB1C3 seq R
5	13	pSB1C3 seq F
5	14	merB seq 1F
5	15	pSB1C3 seq R

Results:.

Conclusion:.

/*----------*/
Page:. 153

Date:. 7/7/14

Title:.

Purpose:.Start a 4 ml culture for pBBRBB-merAB colonies 1 + 2 (plate from 7/2).

Methods:.
Added 4 l km to each tube and left them in the 37^0C incubator overnight in the CSD lab.

Results:.

Conclusion:.

/*----------*/
Page:. 154

Date:. 7/8/14

Title:. Mini-prep of *E. coli* cells

Purpose:. to mini-prep cells and quantify the amount of DNA in the cells

Methods:.
Followed instructions given in kit except 50ul of H_2O and 10 min incubate.

Results:.
49 ul of

Shipping		Characterization	
m1	41.7 (ug/ml)	m5	18.0 (ug/ml)
m2	59.5	l5	10.6
m4	79.1	m4	13.3
l4	18.1	m3	14.8

Conclusion:. Verify orientation of shipping vector.

/*----------*/
Page:. 155

Date:.7/8/14

Title:.

Purpose:. To set up overnight liquid cultures of 3 positives from pBBRBB-merR transformations for mini prep tomorrow and to reattempt merR ligation into pSB1C3 for transformation into *E. coli.*

Methods:.
Follow protocol on page 5-6 using 3 test tubes. Grow overnight on 37^0C shaker.

Follow method on page 89 wit following recipe
2 ul backbone (pSB1C3 48.1 ng/ul)
25 ul insert (merR 3.03 ng/ul)
3 ul 10x T4 Ligase buffer
1 ul T4 DNA ligase
1 ul water

Incubate overnight @ 16^0C

Results:.

Conclusion:.

/*----------*/
Page:. 156

Date:. 7/8/14

Title:. New transformation of pSB1C3::merP into UQ950 *E.coli*

Purpose:.

Methods:.
The transformation was performed following the procedure on page 29.

The transformants were diluted in 1:10, 1:100, and 1:1000 and plated on LB+cm plates. Afterwards they were incubated at 37^0C.

Results:.

Conclusion:.

/*----------*/